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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification 6 :</b> <b>G01N 33/00</b>	<b>A2</b>	<b>(11) International Publication Number:</b> <b>WO 98/53311</b> <b>(43) International Publication Date:</b> 26 November 1998 (26.11.98)
<b>(21) International Application Number:</b> PCT/US98/10520 <b>(22) International Filing Date:</b> 22 May 1998 (22.05.98)  <b>(30) Priority Data:</b> 60/047,488                      23 May 1997 (23.05.97)                      US  <b>(71) Applicant:</b> GAMERA BIOSCIENCE CORPORATION [US/US]; 200 Boston Avenue, Medford, MA 02155 (US).  <b>(72) Inventors:</b> KELLOGG, Gregory; 34 Belknap Street #3, Somerville, MA 02144 (US). KIEFFER-HIGGINS, Stephen, G.; 30 Beaumont Street, Dorchester, MA 02124 (US). CARVALHO, Bruce, L.; 59 Merrill Road, Watertown, MA 02172 (US). DAVIS, Gene, A.; 51 Eldred Street, Lexington, MA 02173 (US). WILLIS, John, P.; 24 Whitney Road, Shirley Center, MA 01464 (US). MINIOR, Ted; 11 Cot Hill Road, Bedford, MA 02155 (US). CHAPMAN, Laura, L.; 87 Marshall Street, Somerville, MA 02145 (US). KOB, Mikayla; 14 Ashford Street, Allston, MA 02134 (US). OELTJEN, Sarah, D.; 46 Hancock Street, Somerville, MA 02144 (US). OMMERT, Shari; 175 Main Street, Medford, MA 02155 (US). MIAN, Alec; 137 Magazine Street, Cambridge, MA 03139 (US).	<b>(74) Agent:</b> NOONAN, Kevin, E.; McDonnell Boehnen Hulber & Berghoff, 32nd floor, 300 South Wacker Drive, Chicago, IL 60606 (US).  <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>	
<b>(54) Title:</b> DEVICES AND METHODS FOR USING CENTRIPETAL ACCELERATION TO DRIVE FLUID MOVEMENT IN A MICROFLUIDICS SYSTEM  <b>(57) Abstract</b>  This invention provides methods and apparatus for performing microanalytic and microsynthetic analyses and procedures. Specifically, the invention provides a microsystem platform for use with a micromanipulation device to manipulate the platform by rotation, thereby utilizing the centripetal force resulting from rotation of the platform to motivate fluid movement through microchannels embedded in the microplatform. The microsystem platforms of the invention are also provided having microfluidics components, resistive heating elements, temperature sensing elements, mixing structures, capillary and sacrificial valves, and methods for using these microsystems platforms for performing biological, enzymatic, immunological and chemical assays. An electronic spindle designed rotor capable of transferring electrical signals to and from the microsystem platforms of the invention is also provided.		

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**Devices and Methods for Using Centripetal Acceleration to Drive Fluid  
Movement in a Microfluidics System**

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**BACKGROUND OF THE INVENTION**

**1. Field of the Invention**

10 This invention relates to methods and apparatus for performing microanalytic and  
microsynthetic analyses and procedures. In particular, the invention relates to  
microminiaturization of genetic, biochemical and chemical processes related to analysis,  
synthesis and purification. Specifically, the invention provides a microsystem platform  
and a micromanipulation device to manipulate the platform by rotation, thereby utilizing  
15 the centripetal forces resulting from rotation of the platform to motivate fluid movement  
through microchannels embedded in the microplatform. The microsystem platforms of  
the invention are also provided having microfluidics components, resistive heating  
elements, temperature sensing elements, mixing structures, capillary and sacrificial  
valves, and methods for using these microsystems platforms for performing biological,  
20 enzymatic, immunological and chemical assays. A slip ring designed rotor capable of  
transferring electrical signals to and from the microsystem platforms of the invention is  
also provided.

**2. Summary of the Related Art**

25 In the field of medical, biological and chemical assays, mechanical and  
automated fluid handling systems and instruments are known in the prior art.

U.S. Patent No. 4,279,862, issued July 21, 1981 to Bertaudiere *et al.* disclose a  
centrifugal photometric analyzer.

U.S. Patent No. 4,381,291, issued April 26, 1983 to Ekins teach analytic  
30 measurement of free ligands.

U.S. Patent No. 4,515,889, issued May 7, 1985 to Klose *et al.* teach automated  
mixing and incubating reagents to perform analytical determinations.

U.S. Patent No. 4,676,952, issued June 30, 1987 to Edelmann *et al.* teach a photometric analysis apparatus.

U.S. Patent No. 4,745,072, issued May 17, 1998 to Ekins discloses immunoassay in biological fluids.

5 U.S. Patent No. 5,061,381, issued October 29, 1991 to Burd discloses a centrifugal rotor for performing blood analyses.

U.S. Patent No. 5,122,284, issued June 16, 1992 to Braynin *et al.* discloses a centrifugal rotor comprising a plurality of peripheral cuvettes.

10 U.S. Patent No. 5,160,702, issued November 3, 1993 to Kopf-Sill and Zuk discloses rotational frequency-dependent "valves" using capillary forces and siphons, dependent on "wettability" of liquids used to prime said siphon.

U.S. Patent No. 5,171,695, issued December 15, 1992 to Ekins discloses determination of analyte concentration using two labelling markers.

15 U.S. Patent No. 5,173,193, issued December 22, 1992 to Schembri discloses a centrifugal rotor for delivering a metered amount of a fluid to a receiving chamber on the rotor.

U.S. Patent No. 5,242,803, issued September 7, 1993 to Burtis *et al.* disclose a rotor assembly for carrying out an assay.

20 U.S. Patent No. 5,409,665, issued April 25, 1995 to Burd discloses a cuvette filling in a centrifuge rotor.

U.S. Patent No. 5,413,009, issued July 11, 1995 to Ekins discloses a method for analyzing analytes in a liquid.

25 U.S. Patent No. 5,472,603, issued December 5, 1995 to Schembri discloses an analytical rotor comprising a capillary passage having an exit duct wherein capillary forces prevent fluid flow at a given rotational speed and permit flow at a higher rotational speed.

Anderson, 1968, *Anal. Biochem.* 28: 545-562 teach a multiple cuvette rotor for cell fractionation.

30 Renoe *et al.*, 1974 *Clin. Chem.* 20: 955-960 teach a "minidisc" module for a centrifugal analyzer.

Burtis *et al.*, 1975, *Clin. Chem.* 20: 932-941 teach a method for a dynamic



introduction of liquids into a centrifugal analyzer.

Fritsche *et al.*, 1975, *Clin. Biochem.* 8: 240-246 teach enzymatic analysis of blood sugar levels using a centrifugal analyzer.

5 Burtis *et al.*, 1975, *Clin Chem.* 21: 1225-1233 teach a multipurpose optical system for use with a centrifugal analyzer.

Hadjioannou *et al.*, 1976, *Clin. Chem.* 22: 802-805 teach automated enzymatic ethanol determination in biological fluids using a miniature centrifugal analyzer.

Lee *et al.*, 1978, *Clin. Chem.* 24: 1361-1365 teach a automated blood fractionation system.

10 Cho *et al.*, 1982, *Clin. Chem.* 28: 1956-1961 teach a multichannel electrochemical centrifugal analyzer.

Bertrand *et al.*, 1982, *Clinica Chimica Acta* 119: 275-284 teach automated determination of serum 5'-nucleotidase using a centrifugal analyzer.

15 Schembri *et al.*, 1992, *Clin Chem.* 38: 1665-1670 teach a portable whole blood analyzer.

Walters *et al.*, 1995, Basic Medical Laboratory Technologies, 3rd ed., Delmar Publishers: Boston teach a variety of automated medical laboratory analytic techniques.

Recently, microanalytical devices for performing select reaction pathways have been developed.

20 U.S. Patent No. 5,006,749, issued April 9, 1991 to White disclose methods apparatus for using ultrasonic energy to move microminiature elements.

U.S. Patent No. 5,252,294, issued October 12, 1993 to Kroy *et al.* teach a micromechanical structure for performing certain chemical microanalyses.

25 U.S. Patent 5,304,487, issued April 19, 1994 to Wilding *et al.* teach fluid handling on microscale analytical devices.

U.S. Patent 5,368,704, issued November 29, 1994 to Madou *et al.* teach microelectrochemical valves.

International Application, Publication No. W093/22053, published 11 November 1993 to University of Pennsylvania disclose microfabricated detection structures.

30 International Application, Publication No. W093/22058, published 11 November 1993 to University of Pennsylvania disclose microfabricated structures for performing

polynucleotide amplification.

Columbus *et al.*, 1987, *Clin. Chem.* 33: 1531-1537 teach fluid management of biological fluids.

5 Ekins *et al.*, 1994 *Ann. Biol. Clin.* 50: 337-353 teach a multianalytic microspot immunoassay.

Wilding *et al.*, 1994, *Clin. Chem.* 40: 43-47 disclose manipulation of fluids on straight channels micromachined into silicon.

One drawback in the prior art microanalytical methods and apparatus has been the difficulty in designing systems for moving fluids on microchips through channels and reservoirs having diameters in the 10-100 $\mu$ m range. Microfluidic systems require  
10 precise and accurate control of fluid flow and valving to control chemical reactions and analyte detection. Conventional pumping and valving mechanisms have been difficult to incorporate into microscale structures due to inherent conflicts-of-scale. These conflicts of scale arise in part due to the fact that molecular interactions arising out of  
15 mechanical components of such components, which are negligible in large (macroscopic) scale devices, become very significant for devices built on a microscopic scale.

Systems that use centripetal force to effect fluid movement in microstructures address the need for a pumping mechanism to effect fluid flow, but cannot alone solve  
20 these scale-related drawbacks of conventional fluidics reduced to microfluidics scale. There remains a need for a simple, flexible, reliable, rapid and economical microanalytic and microsynthetic reaction platform for performing biological, biochemical and chemical analyses and syntheses that can move fluids within the structural components of a microsystems platform. Such a platform should be able to  
25 move nanoliter-to microliter amounts of fluid, including reagents and reactants, at rapid rates to effect the proper mixing of reaction components, removal of reaction side products, and isolation of desired reaction products and intermediates. There remains a need in the art for centripetally-motivated microfluidics platforms capable of precise and accurate control of flow and metering of fluids in both microchip-based and  
30 centrifugal microplatform-based technologies.

### SUMMARY OF THE INVENTION

This invention provides microsystems platforms as disclosed in co-owned and co-pending U.S. Serial No. 08/761,063, filed December 5, 1996 and incorporated by reference herein. Specifically, this invention provides microfluidics components, resistive heating elements, temperature sensing elements, mixing structures, capillary and sacrificial valves, and methods for using these microsystems platforms for performing biological, enzymatic, immunological and chemical assays.

It is an advantage of the centrifugal rotors and microsystems platforms of the invention that an imprecise amount of a fluid comprising a biological sample can be applied to the rotor or platform and a precise volumetric amount of the biological sample is delivered to a fluid reservoir comprising a reaction vessel or other component of the rotor or platform for performing chemical, biochemical, immunological or other analyses. It is an advantage of the centrifugal rotors and microsystems platforms of the invention that metering of said precise amount of a biological fluid sample, for example, a drop of blood, is provided as an intrinsic property of the metering capillary channel of the rotor or platform, thereby avoiding variability introduced by centripetal metering of the sample into a reaction reservoir. It is a further advantage of the centrifugal rotors and microsystems platforms of the invention that an operator can avoid having to precisely measure an amount of a fluid comprising a biological sample for application to the rotor or microsystem platform, thereby permitting end-users, including consumers, having a lower level of sophistication to use a medically diagnostic or other embodiment of the rotor or microsystem platform of the invention.

It is an advantage of the centrifugal rotors and microsystems platforms of the invention that fluid movement into and out of fluid reservoirs on the rotor or platform is precisely determined by displacement of a first fluid, such as biological sample, from a fluid reservoir by a second fluid contained in a second reservoir on the rotor or platform. It is also an advantage of the centrifugal rotors and microsystems platforms of the invention that approximately complete replacement of the volumetric capacity of a first reservoir can be achieved by using fluid displacement as disclosed herein, thereby providing for maximum recovery of a first fluid sample upon displacement by a second fluid, or maximum delivery and replacement of the first fluid by the second fluid. This

aspect of the invention is advantageous for providing sequential chemical or biochemical reaction steps wherein mixing of the reagents is not desired.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

### **DESCRIPTION OF THE DRAWINGS**

Figures 1 through 3 illustrate the microfluidics array of the microsystem platform described in Example 1.

Figures 4 through 6 illustrate the microfluidics array of the microsystem platform described in Example 2.

Figures 7 through 9 illustrate the microfluidics array of the microsystem platform described in Example 3.

Figures 10 through 12 illustrate the microfluidics array of the microsystem platform described in Example 4.

Figures 13 through 15 illustrate the microfluidics array of the microsystem platform described in Example 5.

Figures 16 through 18 illustrate the microfluidics array of the microsystem platform described in Example 6.

Figures 19 through 21 illustrate the microfluidics array of the microsystem platform described in Example 7.

Figures 22 through 24 illustrate the microfluidics array of the microsystem platform described in Example 8.

Figures 25 through 27 illustrate the microfluidics array of the microsystem platform described in Example 9.

Figure 28 illustrates a microfluidics array of a multiplicity of mixing chambers of the microsystem platform described in Example 8.

Figure 29 illustrates the electronic spindle of the invention.

Figure 30 illustrates screen-printing of a resistive heater element as described in Example 10.

Figure 31 illustrates screen-printing of a resistive heater element as described in Example 10.

Figure 32 is a graph showing the time dependence of temperature produced at a variety of voltages using a resistive heater element as described in Example 10.

5        Figure 33 is a graph showing the voltage dependence of temperature produced at a variety of voltages using a resistive heater element as described in Example 10.

Figure 34 is a graph showing the distance dependence on heating using a resistive heater element as described in Example 10.

10       Figure 35 illustrates screen-printing of a resistive heater element associated with wax valves as described in Example 11.

Figure 36 illustrates screen-printing of a resistive heater element associated with wax valves as described in Example 11.

Figure 37 illustrates screen-printing of a resistive heater element associated with wax valves as described in Example 11.

15       Figure 38 illustrates the use of screen-printed resistive heater elements to melt wax valves and control fluid flow in a microfluidics array as described in Example 11.

Figure 39 is a graph showing that temperature can be cycled using a screen-printed resistive heater element as described in Example 14.

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#### **DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS**

The present invention provides centrifugal rotors and microsystems platforms for providing centripetally-motivated fluid micromanipulation.

25       For the purposes of this invention, the term "sample" will be understood to encompass any fluid, solution or mixture, either isolated or detected as a constituent of a more complex mixture, or synthesized from precursor species.

30       For the purposes of this invention, the term "in fluid communication" or "fluidly connected" is intended to define components that are operably interconnected to allow fluid flow between components. In preferred embodiments, the platform comprises a rotatable platform, more preferably a disk, whereby fluid movement on the disk is motivated by centripetal force upon rotation of the disk.

For the purposes of this invention, the term "a centripetally motivated fluid micromanipulation apparatus" is intended to include analytical centrifuges and rotors, microscale centrifugal separation apparatus, and most particularly the microsystems platforms and disk handling apparatus of International Application No. WO97/21090.

5 For the purposes of this invention, the term "microsystems platform" is intended to include centripetally-motivated microfluidics arrays as disclosed in International Application No. WO97/21090.

For the purposes of this invention, the terms "capillary", "microcapillary" and "microchannel" will be understood to be interchangeable and to be constructed of either wetting or non-wetting materials where appropriate.

10.

For the purposes of this invention, the term "fluid chamber" will be understood to mean a defined volume on a rotor or microsystems platform of the invention comprising a fluid.

15

For the purposes of this invention, the term "entry port" will be understood to mean a defined volume on a rotor or microsystems platform of the invention comprising a means for applying a fluid to the rotor or platform.

20

For the purposes of this invention, the term "capillary junction" will be understood to mean a junction of two components wherein one or both of the lateral dimensions of the junction are larger than the corresponding dimensions the capillary. In wetting or wettable systems, the such junctions are where capillary valving occurs, because fluid flow through the capillaries is stopped at such junctions. In non-wetting or non-wettable junctions, the exit from the chamber or reservoir is where the capillary junction occurs. In general, it will be understood that capillary junctions are formed when the dimensions of the components change from a small diameter (such as a capillary) to a larger diameter (such as a chamber) in wetting systems, in contrast to non-wettable systems, where capillary junctions form when the dimensions of the components change from a larger diameter (such as a chamber) to a small diameter (such as a capillary).

25

For the purposes of this invention, the term "biological sample" or "biological fluid sample" will be understood to mean any biologically-derived analytical sample, including but not limited to blood, plasma, serum, lymph, saliva, tears, cerebrospinal

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fluid, urine, sweat, plant and vegetable extracts, semen, and ascites fluid.

For the purposes of this invention, the term "air displacement channels " will be understood to include ports in the surface of the platform that are contiguous with the components (such as chambers and reservoirs) on the platform, and that comprise vents  
5 and microchannels that permit displacement of air from components of the platforms and rotors by fluid movement.

For the purposes of this invention, the term "capillary action" will be understood to mean fluid flow in the absence of rotational motion or centripetal force applied to a fluid on a rotor or platform of the invention.

10 For the purposes of this invention, the term "capillary microvalve" will be understood to mean a capillary comprising a capillary junction whereby fluid flow is impeded and can be motivated by the application of pressure on a fluid, typically by centripetal force created by rotation of the rotor or platform of the invention.

The microplatforms of the invention (preferably and hereinafter collectively referred to as "disks"; for the purposes of this invention, the terms "microplatform",  
15 "microsystems platform" and "disk" are considered to be interchangeable), are provided to comprise one or a multiplicity of microsynthetic or microanalytic systems. Such microsynthetic or microanalytic systems in turn comprise combinations of related components as described in further detail herein that are operably interconnected to  
20 allow fluid flow between components upon rotation of the disk. These components can be fabricated as described below either integral to the disk or as modules attached to, placed upon, in contact with or embedded in the disk. The invention also comprises a micromanipulation device for manipulating the disks of the invention, wherein the disk is rotated within the device to provide centripetal force to effect fluid flow on the disk.  
25 Accordingly, the device provides means for rotating the disk at a controlled rotational velocity, for stopping and starting disk rotation, and advantageously for changing the direction of rotation of the disk. Both electromechanical means and control means, as further described herein, are provided as components of the devices of the invention. User interface means (such as a keypad and a display) are also provided, as further  
30 described in International Application WO97/21090.

Fluid (including reagents, samples and other liquid components) movement is

controlled by centripetal acceleration due to rotation of the platform. The magnitude of centripetal acceleration required for fluid to flow at a rate and under a pressure appropriate for a particular microsystem is determined by factors including but not limited to the effective radius of the platform, the position angle of the structures on the platform with respect to the direction of rotation and the speed of rotation of the platform.

The capillary junctions and microvalves of the invention are based on the use of rotationally-induced fluid pressure to overcome capillary forces. Fluids which completely or partially wet the material of the microchannels (or reservoirs, reaction chambers, detection chambers, etc.) which contain them experience a resistance to flow when moving from a microchannel of narrow cross-section to one of larger cross-section, while those fluids which do not wet these materials resist flowing from microchannels (or reservoirs, reaction chambers, detection chambers, etc.) of large cross-section to those with smaller cross-section. This capillary pressure varies inversely with the sizes of the two microchannels (or reservoirs, reaction chambers, detection chambers, etc., or combinations thereof), the surface tension of the fluid, and the contact angle of the fluid on the material of the microchannels (or reservoirs, reaction chambers, detection chambers, etc.). Generally, the details of the cross-sectional shape are not important, but the dependence on cross-sectional dimension results in microchannels of dimension less than  $500\mu\text{m}$  exhibit significant capillary pressure. By varying the intersection shapes, materials and cross-sectional areas of the components of the microsystems platform of the invention, "valve" are fashioned that require the application of a particular pressure on the fluid to induce fluid flow. This pressure is applied in the disks of the invention by rotation of the disk (which has been shown above to vary with the square of the rotational frequency, with the radial position and with the extent of the fluid in the radial direction). By varying capillary valve cross-sectional dimensions as well as the position and extent along the radial direction of the fluid handling components of the microsystem platforms of the invention, capillary valves are formed to release fluid flow in a rotation-dependent manner, using rotation rates of from 100rpm to several thousand rpm. This arrangement allows complex, multistep fluid processes to be carried out using a pre-determined, monotonic increase in rotational rate.



The theoretical principles underlying the use of capillary junctions and microvalves are disclosed in International Patent Application, Publication No. WO97/\_\_\_\_\_.

5 The instant invention provides microsystems platforms comprising microfluidics components, heating elements, temperature sensing elements, capillary valves, sacrificial  
10 valves and a rotor design for transmitting electrical signals to and from the microsystems platforms of the invention. The invention provides fluidics components for capillary metering of precise amounts of a volume of a fluid sample from the application of a less precise volume of a fluid sample at an entry port on the microsystem platform. These  
15 embodiments of the invention provide for delivery of precise amounts of a sample such as a biological fluid sample without requiring a high degree of precision or accuracy by the operator or end-user in applying the fluid to the platform, and is advantageous in  
20 embodiments of the microsystems platforms of the invention that are used by consumers and other relatively unsophisticated users. The invention also provides laminar flow-dependent replacement of a fluid in a first chamber by a second displacement fluid in a  
25 second chamber on the platform. These embodiments of the invention provide approximately complete replacement of a fluid in one chamber on the platform with fluid from another, and thereby provide means for practicing sequential chemical reactions and other sequential processes on the platform under conditions wherein  
30 mixing of the two fluids is disadvantageous. The invention also provides turbulent flow mixing components, which permit thorough mixing of different fluid components on the platform. In particular, the invention provides mixing chambers fluidly connected with fluid reservoirs containing equal amounts of two or more different fluids or unequal amounts of two or more different fluids. In addition, the invention provides fluid reservoirs fluidly connected with mixing chamber of the invention and shaped to  
determine the relative rate of flow of each of the different fluids into the mixing chamber. In these embodiments, gradients of two fluids differing in viscosity, solute concentration or concentration of suspended particulates can be produced using the mixing chambers of the invention. Such gradients can be transferred to reservoirs on the platform for further analytical manipulations, and can form the basis for controlled testing of concentration-dependent effects of various catalysts, drugs, toxins or other biological or chemical agents.

Platforms of the invention such as disks and the components comprising such platforms are advantageously provided having a variety of composition and surface coatings appropriate for a particular application. Platform composition will be a function of structural requirements, manufacturing processes, and reagent compatibility/chemical resistance properties. Specifically, platforms are provided that are made from inorganic crystalline or amorphous materials, *e.g.* silicon, silica, quartz, inert metals, or from organic materials such as plastics, for example, poly(methyl methacrylate) (PMMA), acetonitrile-butadiene-styrene (ABS), polycarbonate, polyethylene, polystyrene, polyolefins, polypropylene and metallocene. These may be used with unmodified or modified surfaces. Surface properties of these materials may be modified for specific applications. Surface modification can be achieved by silanization, ion implantation and chemical treatment with inert-gas plasmas (*i.e.*, gases through which electrical currents are passed to create ionization). Also provided by the invention are platforms made of composites or combinations of these materials, *for example*, platforms manufactures of a plastic material having embedded therein an optically transparent glass surface comprising for example the detection chamber of the platform. Microplatform disks of the invention are preferably fabricated from thermoplastics such as teflon, polyethylene, polypropylene, methylmethacrylates and polycarbonates, among others, due to their ease of molding, stamping and milling. Alternatively, the disks can be made of silica, glass, quartz or inert metal. A fluid handling system is built by sequential application of one or more of these materials laid down in stepwise fashion onto the thermoplastic substrate. Disks of the invention are fabricated with an injection molded, optically-clear base layer having optical pits in the manner of a conventional compact disk (CD). The disk is a round, polycarbonate disk 120mm in diameter and 100 pm thick. The optical pits provide means for encoding instrument control programming, user interface information, graphics and sound specific to the application and driver configuration. The driver configuration depends on whether the micromanipulation device is a hand-held, benchtop or floor model, and also on the details of external communication and other specifics of the hardware configuration. This layer is then overlaid with a reflective surface, with appropriate windows for external detectors, specifically optical detectors, being left clear on the disk. Other layers of polycarbonate of varying

thickness are laid down on the disk in the form of channels, reservoirs, reaction chambers and other structures, including provisions on the disk for valves and other control elements. These layers can be pre-fabricated and cut with the appropriate geometries for a given application and assembled on the disk. Layers comprising materials other than polycarbonate can also be incorporated into the disk. The composition of the layers on the disk depend in large part on the specific application and the requirements of chemical compatibility with the reagents to be used with the disk. Electrical layers can be incorporated in disks requiring electric circuits, such as electrophoresis applications and electrically-controlled valves. Control devices, such as integrated circuits, laser diodes, photodiodes and resistive networks that can form selective heating areas or flexible logic structures can be incorporated into appropriately wired recesses, either by direct fabrication of modular installation onto the disk. Reagents that can be stored dry can be introduced into appropriate open chambers by spraying into reservoirs using means similar to inkjet printing heads, and then dried on the disk. A top layer comprising access ports and air vents, ports or shafts is then applied. Liquid reagents are then injected into the appropriate reservoirs, followed by application of a protective cover layer comprising a thin plastic film.

The platforms of the invention are preferably provided with a multiplicity of components, either fabricated directly onto the platform, or placed on the platform as prefabricated modules. In addition to the integral components, certain devices and elements can be located external to the platform, optimally positioned on a device of the invention in relation to the platform, or placed in contact with the platform either while rotating or when at rest. Components optimally comprising the platforms of the invention or a controlling device in combination therewith include detection chambers, reservoirs, valving mechanisms, detectors, sensors, temperature control elements, filters, mixing elements, and control systems.

This invention provides microsystems platforms comprising the following components.

#### **1. Microfluidics Components**

The platforms of the invention are provided comprising microfluidics handling

structures in fluidic contact with one another. In preferred embodiments, fluidic contact is provided by capillary or microchannels comprising the surface of the platforms of the invention. Microchannel sizes are optimally determined by specific applications and by the amount of delivery rates required for each particular embodiment of the platforms and methods of the invention. Microchannel sizes can range from 0.1 m to a value close to the 1mm thickness of the platform. Microchannel shapes can be trapezoid, circular or other geometric shapes as required. Microchannels preferably are embedded in a platform having a thickness of about 0.1 to 100mm, wherein the cross-sectional dimension of the microchannels across the thickness dimension of the platform is less than 500 $\mu$ m and from 1 to 90 percent of said cross-sectional dimension of the platform. In these embodiments, which is based on the use of rotationally-induced fluid pressure to overcome capillary forces, it is recognized that fluid flow is dependent on the orientation of the surfaces of the components. Fluids which completely or partially wet the material of the microchannels, reservoirs, detection chambers, etc. (*i.e.*, the components) of the platforms of the invention which contain them experience a resistance to flow when moving from a component of narrow cross-section to one of larger cross-section, while those fluids which do not wet these materials resist flowing from components of the platforms of the invention of large cross-section to those with smaller cross-section. This capillary pressure varies inversely with the sizes of the two components, or combinations thereof, the surface tension of the fluid, and the contact angle of the fluid on the material of the components. Generally, the details of the cross-sectional shape are not important, but the dependence on cross-sectional dimension results in microchannels of dimension less than 500 $\mu$ m exhibit significant capillary pressure. By varying the intersection shapes, materials and cross-sectional areas of the components of the platform of the invention, "valves" are fashioned that require the application of a particular pressure on the fluid to induce fluid flow. This pressure is applied in the disks of the invention by rotation of the disk (which has been shown above to vary with the square of the rotational frequency, with the radial position and with the extent of the fluid in the radial direction). By varying capillary valve cross-sectional dimensions as well as the position and extent along the radial direction of the fluid handling components of the platforms of the invention, capillary valves are formed to

release fluid flow in a rotation-dependent manner, using rotation rates of from 100rpm to several thousand rpm. This arrangement allows complex, multistep fluid processes to be carried out using a pre-determined, monotonic increase in rotational rate.

5 A first example of the microfluidics arrays provided by this invention is shown in Figures 1 through 3. A microsystems platform is provided by the invention that is specifically designed for performing two-step assays. These Figures illustrate an array advantageously used for any two-step analytic process; an antibiotic detection assay is illustrated herein.

10 Such a microsystem platform is illustrated in Figure 1. In the Figure, the arrangement of one assay array 12 on a disk 11 is shown; a multiplicity of such arrays can be advantageously arranged on a microsystems platform, most preferably a disk, of the invention, to provide a multi-use or multi-assay platform.

15 The components of the antibiotic assay array are shown in greater detail in Figure 2. It will be understood by a comparison of Figures 1 and 2 that the center of the platform 11 is at the top of Figure 2, and the edge or lateral extent of the platform is at the bottom of Figure 2, illustrated by a curved line. Rotation of the antibiotic array on platform disks of the invention can be in either direction, although rotation in a consistent, particular direction is preferred. Disc embodiments of the platforms of the invention were fashioned from machined acrylic and injection-molded polycarbonate.

20 The overall disc dimensions include an outer radius of about 6cm and an inner radius of about 0.75cm, wherein the disk is mounted on the spindle of a rotary device. The thickness of the disc ranged from about 0.9mm to about 1.5mm. The working fluid volume for reaction with reagents was about 1-150 $\mu$ L.

25 The components of the antibiotic array are as follows. An entry port 201 having a depth in the platform surface ranging from about 0.25mm to about 6mm and lateral dimensions of from about 0.2cm to about 2cm is constructed on the platform, and designed to accommodate a volume of about 5-100 $\mu$ L. This entry port is fluidly connected with an array of metering capillaries 202 having a square cross-sectional diameter ranging from about 0.02mm to about 1mm and proximal ends rounded with respect to entry port 201; the length of this metering capillary array was sufficient to contain a total volume of about 10-50 $\mu$ L. The entry port is also constructed to be fluidly

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connected with an overflow capillary 203 having a cross-sectional diameter of from about 0.02mm to about 1mm and proximal ends rounded with respect to entry port 201. The overflow capillary is fluidly connected with an overflow chamber 205 having a depth in the platform surface ranging from about 0.02mm to about 5mm, provided that the depth of the overflow chamber 205 is greater than the depth of the overflow capillary 203. Metering capillary 202 is fluidly connected to fluid chamber 204 having a depth in the platform surface ranging from about 0.02mm to about 1mm, provided that the depth of the fluid chamber 204 is greater than the depth of the metering capillary 202. Each of the overflow and fluid chambers is also connected with air ports or air channels, such as 211, that have dimensions ranging from about 0.02mm to about 1mm and permit venting of air displaced by fluid movement on the platform. A capillary junction 212 that is about 0.75mm to 1mm deep is present in the air channel to prevent fluid flow into the air channel. For the purposes of the description of these microfluidics structures, a "capillary junction" is defined in a hydrophilic support material as a pocket, depression or chamber that has a greater depth (vertically within the platform) and/ or a greater width (horizontally within the platform) that the fluid channel to which it is fluidly connected. For liquid with a contact angle less than 90° (such as aqueous solutions on platforms made with most plastics, glass and silica), flow is impeded as the channel cross-section increases at the interface of the capillary junction. The force hindering flow is produced by capillary pressure, which is inversely proportional to the cross sectional dimensions of the channel and directly proportional to the surface tension of the liquid, multiplied by the cosine of the contact angle of the fluid in contact with the material comprising the channel. The factors relating to capillarity in microchannels according to this invention have been discussed in co-owned and co-pending U.S. patent application, Serial No. 08/910,726, filed August 12, 1997, incorporated by reference in its entirety herein.

Entry port 201 is positioned on the platform from 1cm to 20cm from the center of rotation. Metering capillary 202 extends from entry port 201 from about 0.2cm to about 20cm. The extent of the length of overflow capillary 203 is at least about 20% greater than the extent of the length of metering capillary 202. The position of fluid chamber 204 is from about 0.5cm to about 10cm from the center of rotation, and the

position of overflow chamber 205 is thus from about 1.5cm to about 11.5cm from the axis of rotation.

The fluid chamber 204 acts as a capillary barrier that prevents fluid flow from metering capillary 202 at a first, non-zero rotational speed  $f_1$  sufficient to permit fluid flow comprising overflow from the entry port 201 through overflow capillary 203 and into overflow chamber 205. The capillary boundary of fluid chamber 204 is constructed to be overcome at a second rotational speed  $f_2$  (where  $f_2 > f_1$ ). Fluid chamber 204 is fluidly connected to capillary 206 that is from about 0.02mm to about 1mm deep and has a cross-sectional diameter ranging from about 0.02mm to about 1mm. Capillary 206 extends from about 0.1cm to about 20cm from fluid chamber 204 and is connected to holding chamber 207. Holding chamber 207 has a depth in the platform surface from about 0.02mm to about 1mm and greater than the depth of capillary 206. Filling of fluid chamber 204 is motivated by centripetal acceleration and is accompanied by fluid flow through capillary 206 into holding chamber 207. Holding chamber 207 is fluidly connected by way of capillary 208, having a cross-sectional diameter of from about 0.1mm to about 1mm and extending from about 0.2cm to about 10cm from holding chamber 207 and is further connected with read chamber 210. Read chamber 210 has a depth in the platform surface of from about 0.02mm to about 5mm. For optical detection methods, read chamber 210 is of sufficient optical quality to prevent excessive scattering for a diffusely-reflecting medium such as milk. For transparent samples, read chamber 210 comprises a reflector, for example. This may be a diffuse reflector on the side of the chamber opposite to that upon which light is incident or a mirror-like surface. In the case of a diffuse reflector such as paint, a porous material, or any other material which causes diffuse reflection because of its rough surface or porous nature, light incident at a given angle is emitted over a hemisphere with a well-known cosine dependence on angle. In this way the detector may be aligned at an angle not equal to the incident angle, so that specular reflection from the plastic window of the chamber does not enter the detector. Alternatively, the mirror-like surface reflects light at an angle equal to the incident angle. In certain embodiments, a sacrificial valve 213 (as described herein below) is placed as shown in the channel 209.

The use of a platform as disclosed in Figures 1 and 2 is illustrated in Figures 3A through 3J. In the use of this platform an imprecise volume (ranging from 1-150 $\mu$ L of fluid) of a fluid is applied to the entry port 201 (Figure 3A). In embodiments of the platform comprising air displacement channels, the fluid will wick into air channel 211 and be stopped by capillary junction 212. Fluid also wicks into metering capillary 202 and overflow capillary 203. Fluid flows through the metering capillary 202 and overflow capillary 203 at zero rotational speed until the fluid reaches capillary junctions at the junction between metering capillary 202 and fluid chamber 204 and overflow capillary 203 and overflow chamber 205 (Figures 3B and 3C). Metering capillary 202 is constructed to define a precise volume from about 1-150 $\mu$ L of fluid between entry port 201 and the capillary junction at fluid chamber 204, which is designed to be at least the amount of the fluid placed by the user in entry port 201.

After sample loading by a user and filling of metering capillary 202 and overflow capillary 203 at zero rotational speed (which can be performed on the disc separately or with then disc engaged with a spindle of a centrifugal device), the platform is spun at a first rotational speed  $f_1$ , ranging from 100-400rpm; the exact value is dependent on the position of the capillary junction components on the platform. For example, for an entry port 201 having a depth of 0.6mm, metering capillary 202 having dimensions of 0.5mm x 0.5mm in cross-section and a capillary junction between metering capillary 202 and fluid chamber 204 positioned about 2.2-3.8cm from the center of rotation, and overflow capillary 203 having dimensions of 0.5mm x 0.5mm in cross-section and a capillary junction between overflow capillary 203 and overflow chamber 205 positioned about 5.4cm from the center of rotation, this first rotational speed  $f_1$  is equal to about 175rpm for either water or milk.

Due to the greater distance of the end of overflow capillary 203 from the center of rotation than the end of metering capillary 202, fluid flows through overflow capillary 203 into overflow chamber 205 and does not flow from metering capillary 202 into fluid chamber 204 at rotational speed  $f_1$ . The platform is spun until all excess fluid is evacuated from entry port 201 and into overflow chamber 205, except the fluid contained in metering capillary 202 (Figure 3D).

At a second rotational speed  $f_2$ , that is greater than the first rotational speed  $f_1$ ,



typically in the range of 400-520rpm, the precise amount of fluid contained in metering capillary 202 is delivered into fluid chamber 204 (Figures 3E through 3H) For example, for an entry port 201 having a depth of 0.6mm, metering capillary 202 having dimensions of 0.5mm x 0.5mm in cross-section and a capillary junction between metering capillary 202 and fluid chamber 204 positioned about of 2.2-3.8cm from the center of rotation, this second rotational speed is equal to 400rpm for either water or milk. Fluid movement into fluid chamber 204 is accompanied by filling of capillary 206 and holding chamber 207.

In embodiments comprising a sacrificial valve 213 in-line with capillary 208 at position 209 shown in Figure 2, release of the sacrificial valve results in fluid flow into read chamber 210. Sacrificial valves as described above are preferably made of a fungible material that can be removed from the fluid flow path. In preferred embodiments, said sacrificial valves are wax valves and are removed from the fluid flow path by heating, using any of a variety of heating means including infrared illumination and most preferably by activation of heating elements on or embedded in the platform surface as described below. In said embodiments, fluid flow is achieved at rotational speed  $f_2$  with removal of the sacrificial valve.

In embodiments of the platforms of the invention comprising antibiotic arrays as described herein and not containing a sacrificial valve at position 209, capillary 208 preferably fills along with filling of holding chamber 207 until the fluid reaches a capillary junction 209 at the junction between capillary 208 and read chamber 210; in such embodiments, the capillary junction has a depth of from about 0.15mm to about 1mm. At a third rotational speed  $f_3$ , that is greater than the second rotational speed  $f_2$ , typically in the range of >520rpm, the fluid contained in holding chamber 207 is delivered into read chamber 210 (Figures 3I and 3J). For example, for a capillary junction 209 having a depth of 0.75mm, and capillary 208 having dimensions of 0.25mm x 0.25mm in cross-section and the capillary junction positioned from about 3.8cm from the center of rotation, this third rotational speed is equal to 500-800rpm for either water or milk.

More generally, the third rotational speed is proportional to  $1/\sqrt{(R_{\text{outer}} - R_{\text{inner}}) \times \{(R_{\text{outer}} \times R_{\text{inner}})/2 \times \text{the diameter of the capillary}\}}$ , where  $R_{\text{outer}}$  is the dimension of the

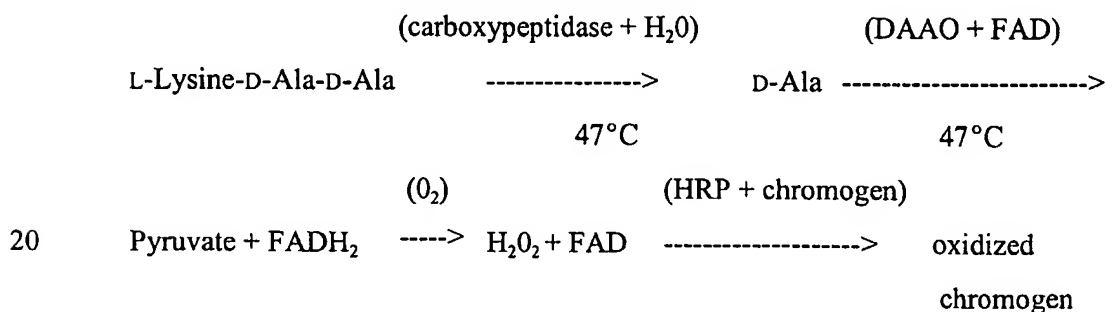
capillary radius at the outer edge and  $R_{inner}$  is the capillary radius at the inner edge, relative to the center of rotation. Thus, for a capillary of square cross-section 0.25mm and a capillary junction 0.75mm deep, values of the product  $\{(R_{outer} - R_{inner}) \times \{(R_{outer} \times R_{inner}) / 2 \times \text{the diameter of the capillary}\}$  ranging from 0.89 to 0.35 yield a third rotational speed of from about 500-800 rpm.

An example of the chemistries of the types of two-step chemical analyses that can be performed using this microfluidics array are illustrated by the antibiotic detection assay. This antibiotic detection assay is performed using the platform design as follows. This chemistry is based on the selective poisoning of an enzyme, carboxypeptidase, by beta lactam antibiotics. Carboxypeptidase is present in a dried form with sugars, buffers or other stabilizers in the holding chamber 207 and a metered amount of milk or other fluid sample solution is introduced into the holding chamber as described above. The milk or other fluid sample solubilizes the enzyme, and the milk/enzyme mixture is incubated at 47° for 3-5 minutes to allow any beta lactam antibiotics present to bind to the carboxypeptidase. The enzyme catalyses cleavage of a D-alanine residue from L-Lysine-D-Ala-D-Ala, and catalysis is inhibited in a concentration-dependent manner by beta-lactam antibiotics present in the sample. In preferred embodiments, this temperature is achieved using a resistive heater element as described below; a description of this resistive heater element is not specifically included in this description of the platform of the invention here in order to focus attention on the disclosed microfluidics structures. It will be understood that any analytic protocol using the microfluidics structures disclosed herein that requires elevated temperatures (*i.e.*, greater than room temperature) advantageously includes resistive heaters as disclosed herein, or other heating elements that are specifically encompassed by the platforms of the invention.

In addition, the holding chamber 207 contains dried reagent comprising a peptide having a D-amino acid at its carboxyl terminus; an example of such a peptide is L-Lysine-D-Ala-D-Ala. Preferably, this peptide is also contained in holding chamber 207 in dried form, and is reconstituted by introduction of milk or other fluid into the chamber as described above. It is an advantage of the microfluidics arrays of the invention that a precisely metered amount of fluid is introduced into holding chamber 207, permitting a standardized amount of carboxypeptidase, peptide substrate and buffers, stabilizers,

etc. be contained in the holding chamber.

In the second step of the assay, D-amino acid oxidase (DAAO), flavine adenine dinucleotide (FAD), horse radish peroxidase (HRP), and a chromogen such as syringalazine (4-hydroxy-3,5-dimethoxybenzaldehyde azine) or ortho-dianisidine (ODA) is contained in dried form in read chamber 210. Displacement of the fluid sample from holding chamber 207 to read chamber 210 results in reconstitution of these reagents, which produce a colored product proportional with the amount of D-amino acid present in the fluid sample after incubation with carboxypeptidase and D-amino acid containing peptide. The D-alanine residue produced by degradation of the peptide substrate by carboxypeptidase in holding chamber 207 is degraded into pyruvate in the presence of DAAO and FAD; the reaction also reduces FAD to FADH<sub>2</sub>. FADH<sub>2</sub> combines with oxygen in the fluid sample to produce hydrogen peroxide and be reoxidized to FADH<sub>2</sub> to FAD. The hydrogen peroxide then acts on a chromogen such as syringalazine in the presence of horse radish peroxidase, and the previously transparent chromogen becomes colored. This reaction scheme is illustrated as follows:



The extent of chromogen production is detected in the read chamber, and related to the presence of antibiotics in the sample by comparison with samples tested in the absence of antibiotic. Most preferably, a standard curve relating the decrease in chromogen production and the amount of antibiotic in the sample is prepared and used to determine the amount of antibiotic in an unknown test sample.

The buffers and reagents used for these chemistries on the platforms of the invention are constituted with appropriate buffers, stabilizers, preservatives, salts, cofactors, adjuvants and other necessary components of the reactions performed on the platform.

An alternative embodiment of the two-step assay microfluidics array is shown in Figures 4 through 6, again exemplified for an antibiotic assay disk of the invention. It will be understood that, as in Example 1, in Figure 4, the arrangement of one assay array 13 on a disk 11 is shown; a multiplicity of such arrays can be advantageously arranged on a microsystems platform, most preferably a disk, of the invention, to provide a multi-use or multi-assay platform.

Disk embodiments of the platforms of the invention were fashioned from machined acrylic. The overall disc dimensions include an outer radius of about 6cm and an inner radius of about 0.75cm, wherein the disk is mounted on the spindle of a rotary device. The thickness of the disc ranged from about 0.9mm to about 1.5mm. The working fluid volume for reaction with reagents was about 25-150 $\mu$ L.

The components of this antibiotic array are as follows. An entry port 301 having a depth in the platform surface of from about 0.25mm to about 5mm and lateral dimensions of from about 0.2cm to about 2cm is constructed on the platform, and designed to accommodate a volume of about 1-100 $\mu$ L. This entry port is fluidly connected with one or a multiplicity of entry capillaries 302 having a square cross-sectional diameter ranging from about 0.02mm to about 1mm and having a depth of 0.1 to 1mm equal to the depth of the entry port, and proximal ends rounded with respect to entry port 301. The length of this metering capillary array was sufficient to contain a total volume of about 20 $\mu$ L. The entry capillaries 302 are fluidly connected to fluid chamber 303 having a depth in the platform surface of from about 0.02mm to about 1mm, wherein the depth is greater than the depth of the entry capillary 302. Each of the fluid chambers of this aspect of the invention is also connected with air ports or air channels, such as 311, that have dimensions ranging from about 0.02mm to about 5mm that permit venting of air displaced by fluid movement on the platform. A capillary junction 312 that is about 0.75mm deep is present in the air channel to prevent fluid flow into the air channel.

The fluid chamber 303 is also constructed to be fluidly connected with an overflow capillary 304 having a cross-sectional diameter of about 0.02mm to about 0.75mm and proximal ends rounded with respect to fluid chamber 304. The overflow capillary is fluidly connected with an overflow chamber 306 having a depth in the

platform surface of from about 0.02mm to about 1mm, greater than the depth of the overflow capillary 304.

Entry port 301 is positioned on the platform from 1cm to 20cm from the center of rotation. Entry capillaries 302 extends from entry port 301 from about 0.5cm to about 10cm. The position of a first fluid chamber 303 is from about 0.5cm to about 10cm from the center of rotation.

The first fluid chamber 303 acts as a capillary barrier that prevents fluid flow from entry capillary 302 at zero rotational speed. Movement of fluid from entry port 301 through entry capillaries 302 and into the first fluid chamber 303 is achieved by rotation at a first, non-zero rotational speed  $f_1$ . Displacement of fluid into the first fluid chamber 303 is accompanied by fluid filling of channel 305 that is fluidly connected with the first fluid chamber 303 and is positioned at the most radially distal point of the first fluid chamber. Channel 305 is fluidly connected with a second fluid chamber 307 and forms a capillary boundary between channel 305 and chamber 307. This capillary boundary is constructed to be overcome at second rotational speed  $f_2$  (where  $f_2 > f_1$ ). First fluid chamber 303 is also fluidly connected to overflow capillary 304 that is from about 0.05mm to about 1mm deep and has a cross-sectional diameter of from about 0.05mm to about 1mm and extends from about 0.2cm to about 20cm. Overflow capillary 304 is connected to overflow chamber 306 that has a depth in the platform surface equal to that of overflow capillary 304, so that there is no capillary boundary between overflow capillary 304 and overflow chamber 306. Overflow capillary 304 is positioned in the first fluid chamber 303 at a point radially less distant from entry port 301 than channel 305, thereby defining a volume in the fluid chamber between the position of the overflow capillary 304 and the most radially distant extent of the said first fluid chamber.

Second fluid chamber 307 is further fluidly connected through channel 308 to a small pocket or capillary junction 309, having a depth in the platform surface of from about 0.1mm to about 5mm and positioned about 0.2 to 20cm from the axis of rotation. Channel 308 has a cross-sectional diameter ranging from about 0.02mm to about 1mm and extends from about 0.2cm to about 10cm, and further extends to a third fluid chamber 310. Third fluid chamber 310 has a depth in the platform surface of from about

0.1mm to about 5mm, that is greater than the depth of capillary 308. Air recirculation channels 311 that have dimensions of from about 0.02mm to about 1mm provide pathways for air displaced by fluid movement, while capillary junctions 312 that are about 0.75mm deep prevent fluid from entering the air channels. In some embodiments of the device a sacrificial valve 313 is placed as shown in the channel 309. In certain  
5       embodiments, a valve 314 is placed in channel 305 to control fluid movement from the first fluid chamber 303 to the second fluid chamber 307.

The use of this platform is illustrated in Figures 6A through 6J. An imprecise volume (ranging from 1-150 $\mu$ L of fluid) of a fluid is applied to the entry port 301  
10       (Figure 6A). Fluid wicks into entry capillary 302 and stops at the capillary junction between entry capillary 302 and the first fluid chamber 303 (Figures 6B and 6C). Fluid flows through the entry capillary B and into the first fluid chamber 303 at a first rotational speed  $f_1$ , ranging from 100-500rpm; the exact value is dependent on the position of the components on the platform (Figures 6D and 6E). For example, for an  
15       entry port 301 having a depth of 0.75mm, entry capillary 302 having dimensions of 0.25mm x 0.5mm in cross-section and a length of 0.5-1cm from the center of rotation, this first rotational speed  $f_1$  is equal to about 250rpm for either water or milk. The fluid further enters capillary channel 305, stopping at the capillary junction with the second fluid chamber 307. As rotation continues, the fluid continues to fill the first fluid  
20       chamber 303, overflow capillary 304 fills (Figure 6F), and excess fluid fills overflow chamber 306 until the level of fluid in the first fluid chamber 303 falls below the position of overflow capillary 304 (Figure 6G).

At a second rotational speed  $f_2$ , that is greater than the first rotational speed  $f_1$ , typically in the range of 100-1000rpm, the capillary junction between channel 305 and  
25       the second fluid chamber 307 is overcome, and fluid remaining in the first fluid chamber 303 is delivered into the second fluid chamber 307 (Figures 6H and 6I). For example, for channel 305 having dimensions of 0.25mm x 0.5mm in cross-section and a length of 2.5-3.3cm from the center of rotation, this second rotational speed is equal to 280rpm for either water or milk.

30       In an alternative embodiment, a sacrificial valve 314 is placed at the junction of channel 305 and the second fluid chamber 308, which sacrificial valve is released to

permit fluid flow through channel 305 and into the second fluid chamber 308. In such embodiments, fluid flow can be achieved at either  $f_1$  or  $f_2$  rotational velocity.

5 In embodiments comprising a sacrificial valve 313 in-line with capillary 308 at position 309 shown in Figure 5, release of the sacrificial valve results in fluid flow into the third fluid chamber 310. Sacrificial valves as described above are preferably made of a fungible material that can be removed from the fluid flow path. In preferred  
10 embodiments, said sacrificial valves are wax valves and are removed from the fluid flow path by heating, using any of a variety of heating means including infrared illumination and most preferably by activation of heating elements on or embedded in the platform surface as described below. In said embodiments, fluid flow is achieved at rotational speed  $f_2$  with removal of the sacrificial valve.

In embodiments of the platforms of the invention comprising antibiotic arrays as described herein and not containing a sacrificial valve at position 310, capillary 309 preferably fills along with filling of the second chamber 308 until the fluid reaches a  
15 capillary junction 309 at the junction between capillary 308 and the third fluid chamber 310; in such embodiments, the capillary junction has a depth of about 0.75mm (ranging from about 0.25mm to about 1mm). At a third rotational speed  $f_3$ , that is greater than the second rotational speed  $f_2$ , typically in the range of >500rpm, the fluid contained in the second chamber 308 is delivered into the third fluid chamber 310 (Figures 6H through  
20 6K). For example, capillary 309 having dimensions of 0.25mm x 0.25mm in cross-section and a length of 3.36-3.7cm from the center of rotation, this third rotational speed is equal to 400rpm for either water or milk.

This embodiment of the platforms of the invention can be used for any two-step analytical assay. Using the antibiotic assay described above as an example, the second  
25 fluid chamber 308 contains a reagent, such as carboxypeptidase, and its substrate, for example L-lysine-D-alanine-D-alanine, and incubation is performed therein to produce D-Ala. The remaining reagents are placed in the third fluid chamber 310, and the reaction including color development advantageously proceeds *in situ* whereby chamber 310 is a read chamber. The extent of chromogen production is detected in the read  
30 chamber, and related to the presence of antibiotics in the sample by comparison with samples tested in the absence of antibiotic. Most preferably, a standard curve relating

the decrease in chromogen production and the amount of antibiotic in the sample is prepared and used to determine the amount of antibiotic in an unknown test sample.

5 The invention also provides microfluidics arrays for separating the fluid component from a particulate suspension. An example of such a particulate suspension is blood, where red and white blood cells are suspended in plasma. Thus, this aspect of the microfluidics embodiments of the invention is illustrated using separation of blood plasma from whole blood.

10 A microsystems platform provided by the invention and specifically designed for separating vertebrate blood cells and components is illustrated in Figures 7 through 9. In Figure 7, the arrangement of one assay array 14 on a disk 11 is shown; a multiplicity of such arrays can be advantageously arranged on a microsystems platform, most preferably a disk, of the invention, to provide a multi-use or multi-assay platform.

15 The components of the blood separation array are shown in greater detail in Figure 8. It will be understood by a comparison of Figures 7 and 8 that the center of the platform 11 is at the top of Figure 8, and the edge or lateral extent of the platform is at the bottom of Figure 8, illustrated by a curved line. Rotation of the blood separation array on platform disks of the invention can be in either direction, although rotation in a consistent, particular direction is preferred. Disk embodiments of the platforms of the invention were fashioned from machined acrylic. The overall disc dimensions include an outer radius of about 6cm and an inner radius of about 0.75cm, wherein the disk is mounted on the spindle of a rotary device. The thickness of the disc ranged from about 0.9mm to about 1.5mm. The working fluid volume was about 1-50 $\mu$ L.

20 The components of the blood separation array are as follows. An entry port 401 having a depth in the platform surface ranging from about 0.1mm to about 5mm and lateral dimensions of about 0.1 to about 2cm is constructed on the platform, and designed to accommodate a volume of about 5 to about 50 $\mu$ L. This entry port is fluidly connected to an entry capillary 402, having a cross-sectional diameter of from about 0.02mm to about 1mm and a depth of about 0.5 to 1mm. The length of this entry capillary was sufficient to contain a total volume of from about 1 to about 15 $\mu$ L. Entry capillary 402 is further fluidly connected to a separation column 403 having a cross-sectional diameter of from about 0.1mm to about 2mm, a depth of from about 0.25mm



to about 1mm, and a length sufficient to contain a total volume of 10 to about 20 $\mu$ L. This separation column is also fluidly connected with a passage 411 to overflow chamber 404. Passage 411 has a cross-sectional diameter ranging from about 0.5mm to about 2mm, a depth of about 0.25mm to about 1mm, and a length of 0.5mm to about 5mm). Overflow chamber 404 has a depth of from about 0.25-1mm.

A small capillary exit 406 is also fluidly connected with separation chamber 403, having a cross-sectional diameter of from about 0.05mm to about 0.25mm, a depth of about 0.025mm to about 0.125mm, and a length about 0.25mm to about 5mm. This capillary is arranged to traverse a direction radially more proximal to the axis of rotation than the insertion point with separation column 403. This small capillary 406 terminates in a capillary junction 407 that is fluidly connected with capillary 408, extending in a radial direction to decant chamber 405. A sacrificial valve 413 is positioned in small capillary 406 at the juncture with the capillary junction. Capillary 408 has a cross-sectional diameter ranging from about 0.05mm to about 1mm, a depth of about 0.05mm to about 1mm, and a length of from about 1mm to about 100mm. This capillary is arranged in a radially outward direction between capillary junction 407 and decant chamber 405. Passage 411 is positioned on separation column 403 to be significantly more proximal to the axis of rotation than the insertion point of small capillary 406.

Air displacement channels 409 that have dimensions of from about 0.02mm to about 1mm permit venting of air displaced by fluid movement on the platform. Capillary junctions 410 that are about 0.75mm deep are present in the air channels to prevent fluid flow into the air channels.

The use of this platform is illustrated in Figures 9A through 9H for separating plasma from whole blood. An imprecise volume (ranging from 1-150 $\mu$ L of fluid) of blood is applied to the entry port 401 (Figure 9A). Blood enters the entry capillary 402 by capillary action, and stops at the capillary junction between entry capillary 402 and the separation chamber 403 (Figures 9B and 9C). At a first rotational speed  $f_1$ , ranging from 100-300rpm (the exact value is dependent on the position of the components on the platform), blood flows from the entry capillary 402 into separation chamber 403 (Figure 9D). Blood continues to fill separation column 403 until blood reaches the position of passage 411, whereupon excess blood flows through passage 411 and into overflow

chamber 404 (Figures 4E and 4F). Advantageously, small channel 406 has dimensions that prevent wicking of blood into the channel as blood flows past the insertion point of small channel 406 into separation column 403.

5 As shown in Figure 9F, after sufficient time of rotation at the first non-zero rotational speed  $f_1$ , the excess blood has been transferred into overflow chamber 404 and the separation column 403 is filled with blood to the position of passage 411. Rotation at a second rotational speed  $f_2$ , that is greater than the first rotational speed  $f_1$ , typically in the range of 1000-5000rpm, blood components are separated into red blood cell, white blood cell (*i.e.*, "buffy coat"), and plasma fractions (Figure 9G). Advantageous  
10 dimensions of small capillary 406 permit fluid flow of the plasma fraction through capillary 406 that is stopped at capillary junction 407. Fluid flow of plasma into decant chamber 405 results from fluid flow overcoming the capillary barrier 407 by rotation at a third rotational speed  $f_3$ , that is greater than the second rotational speed  $f_2$ , typically in the range of >1000-5000rpm (Figure 9H).

15 An alternative embodiment of the fluid separation platform is also provided by the invention, again illustrated by the separation of plasma from whole blood. This embodiment of the blood separation microfluidics array is shown in Figures 10 through 12. It will be understood that, as above, in Figure 10, the arrangement of one separation array 15 on a disk 11 is shown; a multiplicity of such arrays can be advantageously  
20 arranged on a microsystems platform, most preferably a disk, of the invention, to provide a multi-use or multi-assay platform. Disk embodiments of the platforms of the invention are fashioned from machined acrylic. The overall disc dimensions include an outer radius of about 6cm and an inner radius of about 0.75cm, wherein the disk is mounted on the spindle of a rotary device. The thickness of the disc ranged from about 0.9mm  
25 to about 1.5mm. The working fluid volume was about 5-50 $\mu$ L.

The components of this separation array are as follows. An entry port 501 having a depth in the platform surface of about 0.1mm to about 1mm and lateral dimensions of about 0.1cm to about 2cm is constructed on the platform, and designed to accommodate a volume of about 5 to about 50 $\mu$ L. This entry port is fluidly connected  
30 with a first array of metering capillaries 502 and a second array of metering capillaries 503, wherein each of the capillaries has a cross-sectional diameter of from about 0.02mm

to about 1mm. The length of the second metering capillary array 503 is longer than that of the first metering capillary array 502. The first metering capillary array 502 is fluidly connected with a ballast chamber 507, having a depth in the platform surface ranging radially from about 0.1mm to about 5mm and greater than the depth of the first metering capillary array 502, wherein the first metering capillary array 502 forms a capillary junction between the array and the ballast chamber. The second capillary array 503 is fluidly connected with capillary junction 506.

The entry port is also constructed to be fluidly connected with an overflow capillary 504 having a cross-sectional diameter of about 0.02mm to about 1mm and proximal ends rounded with respect to entry port 501. The overflow capillary is fluidly connected with an overflow chamber 505 having a depth in the platform surface of from about 0.1mm to about 5mm, greater than the depth of the overflow capillary 504. Each of the overflow and fluid chambers is also connected with air ports or air channels, such as 514, that have dimensions ranging from about 0.1mm to about 1mm and that permit venting of air displaced by fluid movement on the platform. Capillary junctions 516 that are about 0.75mm deep are present in the air channels to prevent fluid flow into the air channels.

Entry port 501 is positioned on the platform from 0.5cm to 20cm from the center of rotation. Metering capillary array 502 extends from about 0.6cm to about 10cm from entry port 501. Metering capillary array 503 extends about 0.5cm to about 10cm from entry port 501. The length of metering capillary array 503 is about at least about 20% longer than metering capillary array 502, and the extent of the length of overflow capillary 504 is at least about 20% greater than the extent of the length of either the first metering capillary array 502 or the second metering capillary array 503. The position of ballast chamber 507 is about 1cm to about 10cm from the center of rotation, the position of capillary junction 506 is about 1.5 to 15cm from the center of rotation, and the position of overflow chamber 505 is thus about 2.5 to about 20cm from the axis of rotation.

The ballast chamber 507 acts as a capillary barrier that prevents fluid flow from the first metering capillary array 502 at a first, non-zero rotational speed  $f_1$  sufficient to permit fluid flow comprising excess blood overflow from the entry port 501 through

overflow capillary 504 and into overflow chamber 505. Capillary junction 506 is a capillary barrier that prevents fluid flow from the second metering capillary array 503 at said first, non-zero rotational speed  $f_1$  sufficient to permit fluid flow comprising excess blood overflow from the entry port 501 through overflow capillary 504 and into overflow chamber 505. These capillary boundaries are constructed to be overcome at a second rotational speed  $f_2$  (where  $f_2 > f_1$ ).

Ballast chamber 508 is fluidly connected to capillary 510 that is from about 0.02mm to about 1mm deep and has a cross-sectional diameter of about 0.02mm to about 1mm and that extends from about 0.1cm to about 5cm. Capillary 510 is connected to capillary junction 511. Alternatively, capillary 510 is fluidly connected with a sacrificial valve 515. Sacrificial valves as described below are preferably made of a fungible material that can be removed from the fluid flow path. In preferred embodiments, said sacrificial valves are wax valves and are removed from the fluid flow path by heating, using any of a variety of heating means including infrared illumination and most preferably by activation of heating elements on or embedded in the platform surface. In said embodiments, fluid flow is achieved at rotational speed  $f_2$  with removal of the sacrificial valve. Sacrificial valve 515 or capillary junction 511 are further fluidly connected with channel 512 which is from about 0.1mm to about 1mm deep and has a cross-sectional diameter of about 0.1mm to about 1mm. Channel 512 extends about 0.1cm to about 20cm and is fluidly connected with separation chamber 509 at a point most distal from the axis of rotation.

Second metering capillary array 503 is fluidly connected with capillary junction 506, which is overcome at a rotational speed  $f_2 > f_1$ . Capillary junction 506 is further fluidly connected to channel 508, which is further fluidly connected to separation chamber 509. Channel 508 is from about 0.02mm to about 1mm deep and has a cross-sectional diameter of from about 0.02mm to about 1mm. Channel 508 extends from about 0.2cm to about 10cm. Separation chamber 509 is from about 0.2mm to about 5mm deep and has a cross-sectional dimension ranging from about 1mm to about 20mm, and is positioned from about 10mm to about 100mm from the center of rotation.

Separation chamber 509 is fluidly connected with decant channel 517 at a point close to the chamber's most axis-proximal extent. Decant channel 517 is about 0.02mm

to about 1mm deep and has a cross-sectional diameter ranging from about 0.02mm to about 1mm. Decant channel 517 extends from about 4.3cm to about 5cm and is fluidly connected with decant chamber 514. Decant chamber 514 is from about 0.2mm to about 2mm deep and has a cross-sectional diameter of from about 1mm to about 10mm.

5 Decant chamber 514 is positioned about 5.2cm from the center of rotation..

The use of this embodiment of the microfluidics separation arrays of the invention is illustrated in Figures 12A through 12J. An imprecise volume (ranging from 1-150 $\mu$ L of fluid) of blood is applied to the entry port 501 (Figure 12A). Blood enters the each of the metering capillary arrays 502 and 503 and stops at the capillary junction  
10 between metering capillary array 502 and ballast chamber 507 and between metering capillary 503 and capillary junction 506 (Figures 12B and 12C). Blood also enters and fills overflow capillary 504, stopping at the capillary junction with overflow chamber 505.

At a first rotational speed  $f_1$ , ranging from 100-500rpm (the exact value is  
15 dependent on the position of the components on the platform), blood flows from the entry port 501 through overflow capillary 504 and into overflow chamber 505 (Figures 12D and 12E). At a second rotational speed  $f_2$ , that is greater than the first rotational speed  $f_1$ , typically in the range of 300-800rpm, the capillary junction between the first metering capillary array 502 and ballast chamber 508 is overcome, and blood from the  
20 first metering capillary array fills ballast chamber 508 (Figure 12F). Similarly, at second rotational speed  $f_2$ , capillary junction 506 is overcome, and blood from second metering capillary array 503 enters separation chamber 509 (Figure 12F). Advantageously, the volume of blood in second metering capillary array 503 is insufficient to fill separation chamber 509 to the level of insertion of decant channel 517.

By rotation at a third rotational speed  $f_3$ , that is greater than the second rotational speed  $f_2$ , typically in the range of 1000-5000rpm, blood components in separation chamber 509 are separated into red blood cell, white blood cell (*i.e.*, "buffy coat"), and plasma fractions (Figures 12G and 12H). Separation of blood components is not achieved in ballast chamber 507, due to its position on the platform, and the capillary  
25 junction 511 or sacrificial valve 515 are not overcome at third rotational speed  $f_3$ . Advantageously, the separated plasma does not extend to decant capillary 517.

Release of sacrificial valve 517, or rotation at a fourth rotational speed  $f_4$ , that is greater than the third rotational speed  $f_3$ , and typically in the range of 1000-5000rpm, result in flow of blood from ballast chamber 508 through channel 512 and into separation chamber 509 at the "bottom" or most axis-distal extent of the separation chamber (Figure 12I). This results in filling of the separation chamber to a position equal to the insertion point of decant channel 517 (Figure 12J). Plasma flow through decant channel 517 and into decant chamber 514 in an amount equal to the amount of blood contained in ballast chamber 508. Decant channel 517 is advantageously provided with dimensions that retard passage of unfractionated blood, or plasma contaminated with greater than 0.1-1% of blood cells found in whole blood.

The invention also provides mixing chambers and arrays of mixing chambers for mixing two or more fluids that differ in viscosity, solute concentration or concentration of suspended particulates. A first embodiment of microfluidics platform comprising the mixing chambers and arrays of the invention is shown in Figures 13-15 for mixing equal volumes of different liquids. In Figure 13, the arrangement of one assay array 15 on a disk 11 is shown; a multiplicity of such arrays can be advantageously arranged on a microsystems platform, most preferably a disk, of the invention, to provide a multi-use or multi-assay platform.

The components of the mixing array are shown in greater detail in Figure 14. It will be understood by a comparison of Figures 13 and 14 that the center of the platform 11 is at the top of Figure 14, and the edge or lateral extent of the platform is at the bottom of Figure 14, illustrated by a curved line. Rotation of the mixing array on platform disks of the invention can be in either direction, although rotation in a consistent, particular direction is preferred. Disk embodiments of the platforms of the invention were fashioned from machined acrylic. The overall disc dimensions include an outer radius of about 6cm and an inner radius of about 0.75cm, wherein the disk is mounted on the spindle of a rotary device. The thickness of the disc ranged from about 0.9mm to about 1.5mm. The working fluid volume was about 50 $\mu$ L.

The components of the mixing array are as follows. Entry ports 601 having a depth in the platform surface ranging from about 0.1mm to about 1mm and lateral dimensions of from about 1cm to about 5cm are constructed on the platform, and

designed to accommodate a volume of about 5-50 $\mu$ L. Each entry port is fluidly connected with one of a paired array of metering capillaries 602 having a square cross-sectional diameter of from about 0.1mm to about 0.5mm and proximal ends rounded with respect to entry ports 601; the length of each metering capillary array was sufficient to contain a total volume of about 25 $\mu$ L. Metering capillaries 602 are fluidly connected to a curved capillary barrier 603 having a depth in the platform surface of about 0.25mm to about 1mm that is greater than the depth of metering capillaries 602. The capillary barrier 603 and other fluid components of the mixing array are also connected with air channels 608, that have dimensions ranging from about 0.25mm to about 1mm and that permit venting of air displaced by fluid movement on the platform. In addition, capillary junctions 609 that are about 0.75mm deep are present in the air channels to prevent fluid backflow into the air channel.

Capillary barrier 603 is fluidly connected by a narrow capillary channel 604 to mixing chamber 605, which is fluidly connected with channel 610, which is further connected with mixed fluid receiving chamber 606. Alternatively, capillary 604 comprises a sacrificial valve 612. Sacrificial valves used in this embodiment of the invention are as described below. Capillary channel 604 ranges from about 0.1mm to about 1mm in depth and has a cross-sectional diameter of from about 0.1mm to about 1mm). Capillary channel 604 extends from about 0.1 to about 10 cm. Mixing chamber 605 is about 0.1mm to about 1mm deep and has a cross-sectional diameter of from about 0.5mm to about 5mm), is positioned about 1cm to about 30cm from the center of rotation. Capillary channel 610 ranges from about 0.1mm to about 1mm deep and has a cross-sectional diameter of from about 0.1mm to about 1mm). Capillary channel 610 extends from about 0.2cm to about 30cm. In an advantageous embodiment, mixing chamber 605 is constructed such that the insertion point of capillary channel 604 and the insertion point of capillary channel 610 are offset at opposite ends of the mixing chamber. As a consequence, fluid flowing through capillary channel 604 is forced to encounter the opposite wall of mixing chamber 605 before fluid flow can proceed through capillary channel 610. This results in the creation of turbulence in the mixed laminar fluid stream in capillary channel 604 caused by the conjoint flow of fluid from the first and second metering channels without appreciable mixing. The turbulence

created by the structure of mixing chamber 605 is sufficient to disrupt laminar flow and cause fluid mixing in the chamber prior to continued fluid flow through capillary channel 610 and into mixed fluid receiving chamber 606. Alternatively, the positions of the capillaries 604 and 610 can be at any convenient position in mixing chamber 605, wherein the Coriolis forces of fluid flow are sufficient to disrupt laminar flow and provide turbulence that results in efficient mixing.

Mixed fluid receiving chamber 606 is about 0.1mm to about 5mm deep, has a cross-sectional diameter of from about 1mm to about 20mm, and is positioned from about 1cm to about 30cm from the center of rotation.

The use of this embodiment of the invention for mixing equal volumes of fluid (ranging from 1-150 $\mu$ L is illustrated in Figures 15A through 15D. Equal volumes of each fluid to be mixed is applied to the entry ports 601 (Figure 15A). Fluid enters the each of the metering capillary arrays 602 and stops at capillary barrier 603.

At a first rotational speed  $f_1$ , ranging from 50-500rpm (the exact value is dependent on the position of the components on the platform), the fluids from each capillary array flows into and fills the capillary barrier 603 (Figure 15B). In embodiments comprising a sacrificial valve 612, the valve prevents fluid flow into channel 604; otherwise, fluid flow proceeds into channel 604 at rotational speed  $f_1$ . Upon release of sacrificial valve 612, fluid flow proceeds from capillary junction 603 through channel 604 and into mixing chamber 605 (Figure 15C). Fluid flow within mixing chamber 605 is turbulent, in contrast to fluid flow through capillary barrier 603 or channel 604, which is primarily laminar, so that mixing occurs predominantly in mixing chamber 605. Fluid flow proceeds through channel 610 and the mixed fluid solution is displaced into mixed fluid receiving chamber 606 (Figure 15D).

The invention also provides mixing arrays for mixing unequal volumes of fluid. One example of such an additional embodiment of the microsystems platform provided by the invention and specifically designed for performing mixing of unequal volumes of different liquid samples is illustrated in Figures 19 through 21. In Figure 19, the arrangement of one assay array 17 on a disk 11 is shown; a multiplicity of such arrays can be advantageously arranged on a microsystems platform, most preferably a disk, of the invention, to provide a multi-use or multi-assay platform.



The components of the mixing array are shown in greater detail in Figure 20. It will be understood by a comparison of Figures 19 and 20 that the center of the platform 11 is at the top of Figure 20, and the edge or lateral extent of the platform is at the bottom of Figure 20, illustrated by a curved line. Rotation of the mixing array on platform disks of the invention can be in either direction, although rotation in a consistent, particular direction is preferred. Disk embodiments of the platforms of the invention are fashioned from machined acrylic. The overall disc dimensions include an outer radius of about 6cm and an inner radius of about 0.75cm, wherein the disk is mounted on the spindle of a rotary device. The thickness of the disc ranged from about 0.9mm to about 1.5mm. The working fluid volume is about 2-200 $\mu$ L.

The components of this mixing array are as follows. Fluid reservoirs 701 and 702, each containing one of a pair of liquids to be mixed, are constructed on the platform, having a depth in the platform surface ranging from about 0.1mm to about 5mm and lateral dimensions of from about 0.2cm to about 10cm). In this embodiment, fluid reservoir 701 is designed to accommodate a volume of about 1 to about 500 $\mu$ L, and fluid reservoir 702 is designed to accommodate a volume of about ranging from about 1 to about 500 $\mu$ L, wherein the volume of fluid reservoir 702 is less than the volume of fluid reservoir 701. In particular and in addition, the viscosity of the fluid in the fluid reservoirs may differ, so that mixing produces a mixed fluid of intermediate viscosity. Also, in this embodiment the concentration of solute of suspended particulate may differ between the fluids. Each fluid reservoir is fluidly connected with a capillary channel 703 or 704 to capillary junction 705. Each capillary channel is from about 0.02mm to about 1mm deep, has a cross-sectional diameter ranging from about 0.1mm to about 1mm, and extends about 2cm to about 100cm. Capillary junction 705 has a depth in the platform surface ranging from about 0.02mm to about 1mm that is greater than the depth of capillaries 703 to 704. Alternatively, capillaries 703 or 704 comprise a sacrificial valve 712. Sacrificial valves used in this embodiment of the invention are as described below. Use of said sacrificial valves can be used in addition to or in place of capillary junction 705.

The fluid components of the mixing array are also connected with air channels 710, that have dimensions ranging from about 0.1mm to about 1mm and permit venting

of air displaced by fluid movement on the platform. In addition, capillary junctions 711 that are about 0.75mm deep are present in the air channels to prevent fluid backflow into the air channel.

Capillary junction 705 is fluidly connected by a narrow capillary channel 706 to mixing chamber 707, which is fluidly connected with channel 708, which is further connected with mixed fluid receiving chamber 709. Alternatively, capillary 706 comprises a sacrificial valve 712, as described below. Capillary channel 706 ranges from about 0.1mm to about 1mm, has a cross-sectional diameter of from about 0.1mm to about 1mm and extends from about 0.2cm to about 30cm. Mixing chamber 707 is from about 0.1mm to about 1mm deep, has a cross-sectional diameter ranging from about 0.1mm to about 1mm, and is positioned from about 0.2cm to about 30cm from the center of rotation. Capillary channel 708 ranges from about 0.1mm to about 1mm, has a cross-sectional diameter ranging from about 1mm to about 20mm and extends from about 0.2cm to about 30cm. Capillary channel 706 and capillary channel 708 are advantageously offset in their connection with the mixing chamber as described above, or are positioned at any convenient position in the mixing chamber for those embodiments relying on Coriolis forces to create mixing.

Capillary 708 is fluidly connected with mixed fluid receiving chamber 709. Mixed fluid receiving chamber 709 is about 0.1mm to about 1mm, has a cross-sectional diameter of about 1mm to about 20mm, and is positioned from about 1cm to about 30cm from the center of rotation.

The use of this embodiment of the microfluidics components of the invention is illustrated in Figures 21A through 21E. A volume (ranging from 1-150 $\mu$ L of fluid) of each of the fluids to be mixed is applied to the fluid reservoirs 701 and 702 (Figure 21A). Fluid enters the each of the capillaries 703 and 704 and stops at capillary junction 705. Alternatively, the platforms of the invention are provided containing the fluids to be mixed already in fluid reservoirs 701 and 702. In these embodiments, it is preferred that sacrificial valves 712 be provided in capillaries 703 and 704, to prevent evaporation, wetting or leakage of fluid from the reservoirs prior to use.

At a first rotational speed  $f_1$ , ranging from 100-1000rpm (the exact value is dependent on the position of the components on the platform), the fluids from each

capillary flows past capillary junction 605 and through mixing chamber 707 (Figures 21B and 21C). In embodiments comprising a sacrificial valve 712, the valve prevents fluid flow into channels 703 and 704. Upon release of sacrificial valve 712, fluid flow proceeds from capillary junction 705 through channel 706 and into mixing chamber 707 (Figure 21C). Fluid flow within mixing chamber 707 is turbulent, in contrast to fluid flow through capillary barrier 705 or channel 706, which is primarily laminar, so that mixing occurs predominantly in mixing chamber 707. Fluid flow proceed through channel 708 and the mixed fluid solution is displaced into mixed fluid receiving chamber 709 (Figures 21D and 21E).

10 In another embodiment of the mixing chamber arrays of the invention are provided platforms capable of forming a gradient of two or more liquids that differ in viscosity, solute concentration or concentration of suspended particulates. Such an additional embodiment of the microsystems platform provided by the invention and specifically designed for performing mixing of different volumes of liquid samples to form a gradient in the concentration of a species in which the two fluids differ is illustrated in Figures 22-24. In Figure 22, the arrangement of one assay array 18 on a disk 11 is shown; a multiplicity of such arrays can be advantageously arranged on a microsystems platform, most preferably a disk, of the invention, to provide a multi-use or multi-assay platform.

20 The components of the mixing array are shown in greater detail in Figure 23. It will be understood by a comparison of Figures 22 and 23 that the center of the platform 11 is at the top of Figure 23, and the edge or lateral extent of the platform is at the bottom of Figure 23, illustrated by a curved line. Rotation of the mixing array on platform disks of the invention can be in either direction, although rotation in a consistent, particular direction is preferred. Disk embodiments of the platforms of the invention are fashioned from machined acrylic. The overall disc dimensions include an outer radius of about 6cm and an inner radius of about 0.75cm, wherein the disk is mounted on the spindle of a rotary device. The thickness of the disc ranged from about 0.9mm to about 1.5mm. The working fluid volume is about 40 $\mu$ L.

30 The components of the mixing array are as follows. Fluid reservoirs 801 and 802, each containing one of a pair of liquids to be mixed, are constructed on the

platform, having a depth in the platform surface from about 0.1mm to about 5mm and lateral dimensions ranging from about 1cm to about 10cm. Fluid reservoir 801 is designed to accommodate a volume of from about 1 to about 500 $\mu$ L, and fluid reservoir 802 is designed to accommodate a volume of about 1 to about 500 $\mu$ L, wherein the shape of fluid reservoir 802 is different than the shape of fluid reservoir 801. In particular and in addition, fluid reservoirs 801 and 802 are shaped so that the rate of fluid output in the two reservoirs differs between the reservoirs at a particular rotational speed, due to a change in the pressure "head" (related to the cross-sectional area of the fluid at each point in the reservoir), so that the proportion of fluid in the mixture from one of the reservoirs is at a maximum at the beginning of rotation and is at a minimum when the fluids from the reservoirs are completely mixed at the end of rotation, thus forming a gradient. Gradients produced according to this aspect of the invention can consist of salt gradients, including sodium chloride and cesium chloride or sulfate gradients, gradients of low molecular weight species such as sucrose, synthetic polymer gradients such as Ficoll or Hypaque, or gradients of a drug, toxin, enzyme substrate or other small molecule of interest.

Each fluid reservoir is fluidly connected with a capillary channel 803 or 804 to capillary junction 805. Each capillary channel ranges from about 0.1mm to about 1mm deep, has a cross-sectional diameter of from about 0.1mm to about 1mm and extends about 2cm to about 100cm. Capillary junction 805 has a depth in the platform surface of from about 0.1mm to about 1mm that is greater than the depth of capillaries 803 to 804. Alternatively, capillaries 803 or 804 comprise a sacrificial valve 812, as described below. Use of said sacrificial valves can be used in addition to or in place of capillary junction 805.

The fluid components of the mixing array are also connected with air channels 810, that have dimensions of from about 0.1mm to about 1mm and permit venting of air displaced by fluid movement on the platform. In addition, capillary junctions 811 that are about 0.75mm deep are present in the air channels to prevent fluid backflow into the air channel.

Capillary junction 805 is fluidly connected by a narrow capillary channel 806 to mixing chamber 807, which is fluidly connected with channel 808, which is further

connected with mixed fluid receiving chamber 809. Alternatively, capillary 806 comprises a sacrificial valve 812. Capillary channel 806 is from about 0.1mm to about 1mm, has a cross-sectional diameter of from about 0.1mm to about 1mm and extends from about 0.2cm to about 30cm. Mixing chamber 807 is from about 0.1mm to about 1mm, has a cross-sectional diameter of from about 0.1mm to about 1mm, and is positioned from about 0.2cm to about 30cm from the center of rotation. Capillary channel 808 is from about 0.1mm to about 1mm, has a cross-sectional diameter of about 1mm to about 20mm and extends from about 0.2cm to about 30cm. Capillary channel 806 and capillary channel 808 are advantageously offset in their connection with the mixing chamber as described above, or are positioned at any convenient position in the mixing chamber for those embodiments relying on Coriolis forces to create mixing.

Capillary 808 is fluidly connected with mixed fluid receiving chamber 809. Mixed fluid receiving chamber 809 is about 0.75mm deep (ranging from about 0.1mm to about 1mm) and having a cross-sectional diameter of about 5mm (ranging from about 1mm to about 20mm) from about, and is positioned from about 1cm to about 30cm from the center of rotation.

Use of this microfluidics platform to produce a gradient as described herein is illustrated in Figures 24A through 24E. A volume (ranging from 1-150 $\mu$ L of fluid) of each of the fluids to be mixed is applied to the fluid reservoirs 801 and 802 (Figure 24A). Fluid enters the each of the capillaries 803 and 804 and stops at capillary junction 805. Alternatively, the platforms of the invention are provided containing the fluids to be mixed already in fluid reservoirs 801 and 802. In these embodiments, it is preferred that sacrificial valves 812 be provided in capillaries 803 and 804, to prevent evaporation, wetting or leakage of fluid from the reservoirs prior to use.

At a first rotational speed  $f_1$ , ranging from 100-1000rpm (the exact value is dependent on the position of the components on the platform), the fluids from each capillary flows past capillary junction 805 and through mixing chamber 807 (Figures 24B and 24C). In embodiments comprising a sacrificial valve 812, the valve prevents fluid flow into channels 803 and 804. Upon release of sacrificial valve 812, fluid flow proceeds from capillary junction 805 through channel 806 and into mixing chamber 807 (Figure 24D). Fluid flow within mixing chamber 807 is turbulent, in contrast to fluid

flow through capillary barrier 805 or channel 806, which is primarily laminar, so that mixing occurs predominantly in mixing chamber 807. Fluid flow proceed through channel 808 and the mixed fluid solution is displaced into mixed fluid receiving chamber 809 (Figures 24D and 24E).

5           In addition to the embodiments described herein, the invention provides microfluidics arrays comprising a multiplicity of mixing chambers fluidly connected in series to one another. Such an arrangement is illustrated in Figure 28. In particular, mixing cascades as shown in Figure 28 are useful for mixing liquids of dramatically different volumes or viscosities, such as mixing a low volume, high viscosity liquid with  
10           a high volume, low viscosity liquid. In these embodiments, the mixing array comprises a multiplicity of mixing chambers arrayed radially across the platform surface, each mixing chamber having an inlet capillary extending radially from a position more proximal to the center of rotation than the mixing chamber, and an outlet capillary extending radially to a position more distal to the center of rotation than the mixing  
15           chamber. In an advantageous embodiment of this array, the capillaries are connected with the mixing chamber so that their positions in the mixing chamber are offset from one another, wherein fluid flow from the inlet capillary impinges on a wall of the mixing chamber at a position other than the position occupied by the outlet capillary, thereby producing turbulent flow within the mixing chamber that mixes the fluids.  
20           Alternatively, the capillaries can be positioned in the mixing chamber at any convenient position, and Coriolis forces have be relied upon to facilitate mixing. In either type of array, the inlet capillary of the first mixing chamber is fluidly connected with the fluid reservoirs (either directly or through a capillary junction), and the inlet capillary of the other mixing chamber in the array is the outlet channel of the mixing chamber  
25           immediately more proximal to the center of rotation; similarly, the outlet capillary of each mixing chamber is the inlet capillary of the mixing chamber immediately more distal to the center of rotation, and wherein the outlet capillary of the mixing chamber positioned the most distal from the center of rotation is fluidly connected with a mixed fluid receiving chamber. Thus, the fluid is repetitively mixed in a multiplicity of mixing  
30           chambers arrayed more and more distally from the center of rotation, ending with a reservoir or chamber sufficient in volume to accommodate the volume of the mixed fluid. The dimensions of the capillaries, fluid reservoirs, mixing chambers and mixed

fluid receiving chamber in these arrays are as described above.

Figure 28 also illustrates another embodiment of the mixing arrays of the invention. In this embodiment, used in conjunction with the gradient-forming embodiment described above, a specialized mixed fluid receiving chamber, termed a gradient chamber, is provided; one embodiment of this receiving chamber is shown in Figure 28. This chamber permits the gradient fluid stream to be aliquotted into the individual compartments of the chamber, wherein the concentration of the gradient decreases with increasing distance from the gradient chamber inlet capillary. With gradients constituting decreasing concentrations of an analyte, drug, toxin or other species to be tested, the chamber can be modified to contain a detection means in each compartment, so that the concentration effect of the changing component of the gradient can be determined.

In yet another embodiment of the microfluidics platforms of this invention are provided a microsystems platform specifically designed for performing a specific binding assay. These embodiments are exemplified using an immunoassay as illustrated in Figures 25 through 27. In Figure 25, the arrangement of one assay array 19 on a disk 11 is shown; a multiplicity of such arrays can be advantageously arranged on a microsystems platform, most preferably a disk, of the invention, to provide a multi-use or multi-assay platform.

The components of the mixing array are shown in greater detail in Figure 26. It will be understood by a comparison of Figures 25 and 26 that the center of the platform 11 is at the top of Figure 26, and the edge or lateral extent of the platform is at the bottom of Figure 26, illustrated by a curved line. Rotation of the mixing array on platform disks of the invention can be in either direction, although rotation in a consistent, particular direction is preferred. Disk embodiments of the platforms of the invention were fashioned from machined acrylic. The overall disc dimensions include an outer radius of about 6cm and an inner radius of about 0.75cm, wherein the disk is mounted on the spindle of a rotary device. The thickness of the disc ranged from about 0.9mm to about 1.5mm. The working fluid volume for reaction was about 10-100 $\mu$ L.

In this aspect of the platforms of the invention there is provided an incubation chamber that comprises a specific binding reagent. For the purposes of this invention,

the term "specific binding reagent" is intended to encompass biomolecules having a specific binding affinity between pairs thereof providing a specific molecular binding interaction with a binding affinity constant of between about  $10^{-4}$  and  $10^{-15}$ M. Examples of such pairs of specific binding reagents include but are not limited to antigen and antibody, including antisera, polyclonal antibodies and most preferably monoclonal antibodies; receptor and ligands, including cell-surface receptors; integrins and adhesion proteins, including ICAM-I and ICAM-II; and carbohydrates and lectins, including phytohemagglutinin. As provided by the invention, specific binding reagents comprising a first member of a specific binding pair is provided in an incubation chamber as a coating on the surface of the chamber, as a dried or lyophilized reagent contained in the chamber and reconstituted upon addition of a fluid or fluid sample to the chamber; contained on a support media, such as latex or other beads, or in a gel or other support media. Said first member of a specific binding pair is designed or intended to detect the presence of an analyte, for example, a cell expressing a cognate antigen, receptor or adhesion protein or having a carbohydrate moiety at the cell surface specific for a particular lectin. Said specific binding reagent is applied to the incubation chamber of the platform by depositing the reagent on the surface using any appropriate means, including inkjet printing, computer-positioned syringes, microetching and microlithographic methods, including photolithography, screen and airbrush printing methods, solution coating, dipping, and conventional microtitre-well techniques. In applying said specific binding reagent, the surface or detection chamber can be treated to provide a two-dimensional array or pattern, wherein certain areas on the surface or detection chamber are treated with said specific binding reagent and others are not in a recognizable manner.

The components of the specific binding assay array are as follows. An entry port 901 having a depth in the platform surface ranging from about 0.1mm to about 1mm deep and lateral dimensions of about 0.1cm to about 2cm is constructed on the platform, and designed to accommodate a volume of about from  $2\mu\text{L}$  to  $100\mu\text{L}$ . This entry port is fluidly connected with a metering capillary 902 having cross-sectional diameter of from about 0.1mm to about 1mm, and having a depth of about 0.25mm to 1mm; the length of this metering capillary was sufficient to contain a total volume of about 2 to



about 100 $\mu$ L. The metering capillary 902 is fluidly connected to capillary junction 904.

The entry port is also constructed to be fluidly connected with an overflow capillary 903 having a cross-sectional diameter of about 0.1mm to about 1mm and proximal ends rounded with respect to entry port 901. The overflow capillary is fluidly  
5 connected with an overflow chamber 905 having a depth in the platform surface of about 0.1mm to about 1mm, greater than the depth of the overflow capillary 903. Each of the overflow and fluid chambers is also connected with air ports or air channels, such as 523, that have dimensions of about 0.1mm to about 1mm and that permit venting of air displaced by fluid movement on the platform. Capillary junctions 524 that are about  
10 0.75mm deep are present in the air channels to prevent fluid flow into the air channels.

Entry port 901 is positioned on the platform from 1cm to 20cm from the center of rotation. Metering capillary 902 extends from entry port 901 from about 0.5cm to about 5cm. Overflow capillary 902 extends from entry port 901 from about 1cm to about 20cm. The extent of the length of overflow capillary 903 is 20% longer than  
15 metering capillary 902. The position of overflow chamber 905 is from about 1cm to about 20cm from the center of rotation, and the position of capillary junction 904 is from about 1cm to about 20cm from the center of rotation.

Capillary junction 904 is fluidly connected with capillary channel 906, which in turn is fluidly connected with incubation chamber 910. Capillary channel 906 has a  
20 cross-sectional diameter of about 0.1mm to about 1mm and extends from about 0.2cm to about 10cm from the capillary junction. Incubation chamber 910 has a depth in the platform surface ranging from about 0.1mm to about 1mm, that is greater than the depth of capillary channel 906. Capillary channel 906 is also fluidly connected with channel 909 through capillary junction 907. Capillary junction 907 is constructed to prevent  
25 fluid flow backwards through the junction. Channel 909 has a cross-sectional diameter of about 0.1mm to about 1mm and extends from about 0.2cm to about 5cm from the capillary junction. Capillary junction 907 has a depth in the platform surface of about 0.1mm to about 1mm, greater than the depth of the channel 909 or capillary channel 906. Incubation chamber 910 also contains a specific binding species, most preferably an  
30 antibody, specific for a component of the sample. This species is advantageously contained within incubation chamber 910 as a coating on the surface of the chamber, or

attached to beads or other carrier within the chamber, or to a functionalized inner surface of the chamber or otherwise as described above.

Capillary junction 907 is further fluidly connected with wash buffer reservoir 516, having a depth in the platform surface of from about 0.1mm to about 1mm and positioned at a distance from about 10mm to about 200mm from the axis of rotation.

Capillary junction 907 is further fluidly connected with reagent capillary 920, which is further fluidly connected with capillary junction 914, which is further fluidly connected with channel 926, and which is fluidly connected with reagent reservoir 917. Reagent capillary 920 has a cross-sectional diameter of about 0.1mm to about 1mm and extends from about 0.2cm to about 20cm reagent reservoir 917. Capillary junction 914 has a depth in the platform surface ranging from about 0.1mm to about 1mm and is positioned at a distance from about 10mm to about 200mm from the axis of rotation.

Reagent capillary 926 has a cross-sectional diameter of from about 0.1mm to about 1mm and extends from about 0.2cm to about 20cm from the capillary junction. Reagent reservoir 917 has a depth in the platform surface of about 0.1mm to about 1mm, and is positioned at a distance from about 10mm to about 200mm from the axis of rotation.

Incubation chamber 910 is fluidly connected at a point most distal to the axis of rotation to U-shaped capillary 921. U-shaped capillary 921 has a cross-sectional diameter of about 0.1mm to about 1mm and extends from about 0.2cm to about 20cm between incubation chamber 910 and waste reservoir 915. This capillary extends in a U-shape to a point that is at least as proximal to the axis of rotation than the most axis-proximal extent of incubation chamber 910. This positioning of the U-shaped channel relative to incubation chamber 910 ensures that additional fluids flowing into incubation chamber 910 and displacing fluid therefrom will displace said fluid homogeneously, *i.e.*, the first fluid in the chamber will be pushed out of the chamber whilst being replaced by the second fluid.

This U-shaped capillary is also fluidly connected with waste reservoir 915. Waste reservoir 915 has a depth in the platform surface of about 0.1mm to about 5mm and is positioned at a distance from about 10mm to about 200mm from the axis of rotation; as shown in Figure 26, the waste reservoir is typically positioned at the farthest distance from the axis of rotation of any of the components of the array.

In certain embodiments of the invention, sacrificial valves 922 can be positioned at the junction of capillary junction 904 and capillary channel 906, at the junction of capillary junction 907 and wash buffer capillary 908, or at the junction of reagent reservoir 918 and capillary junction 919.

5           The use of this platform for performing an immunoassay is illustrated in Figures 27A through 27L. In the use of this platform reagent reservoir 916 and wash reservoir 915 are pre-loaded on the disk, and most preferably the disk contains sacrificial valves 922 at the junction of capillary junction 907 and wash buffer capillary 908, and at the junction of reagent reservoir 918 and capillary junction 919. An imprecise volume  
10 (ranging from 1-150 $\mu$ L of fluid) of a fluid is applied to the entry port 901 (Figure 27A). Fluid wicks into metering capillary 902 and stops at the capillary junction between metering capillary 902 and capillary junction 904 (Figures 27B and 27C). After sample loading by a user and filling of metering capillary 902 and overflow capillary 903 at no rotational speed, the platform is spun at a first rotational speed  $f_1$ , ranging from 100-  
15 500rpm; the exact value is dependent on the position of the components on the platform.

Due to the greater distance of the end of overflow capillary 903 from the center of rotation than the end of metering capillary 902, fluid flows through overflow capillary 903 into overflow chamber 905 (Figure 27D). The platform is spun until all excess fluid is evacuated from entry port 901 and into overflow chamber 905, except the fluid  
20 contained in metering capillary 902 (Figure 27E).

At a second rotational speed  $f_2$ , that is greater than the first rotational speed  $f_1$ , typically in the range of 100-1000rpm, the capillary junction 904 at the distal end of the metering capillary 902 is overcome, and sample from metering capillary 902 fills incubation chamber 910 (Figures 27F and 27G). A portion of the sample wicks into U-  
25 shaped capillary 914 to the level of the sample in incubation chamber 910 (Figure 27G). The sample is incubated for a time sufficient for maximum saturation binding of the component in the sample that specifically bind to the specific binding species.

At a third rotational speed  $f_3$ , that is greater than the second rotational speed  $f_2$ , typically in the range of 100-1500rpm, the capillary junction 908 is overcome, and wash  
30 buffer from reservoir 916 flows through capillary 909, capillary 906, and into incubation chamber 910. Wash buffer fluid flow forces the sample through U-shaped capillary 914

and into waste reservoir 915 (Figures 27 H through 27J). Preferably, sacrificial valves 922 are released to permit wash buffer fluid flow.

At a fourth rotational speed  $f_4$ , that is greater than the third rotational speed  $f_3$ , typically in the range of 100-2000rpm, the capillary junction 919 is overcome, and reagent buffer from reservoir 917 flows through capillary 918, capillary 920, capillary junction 908, capillary 906, and into incubation chamber 910. Reagent buffer fluid flow forces the wash buffer through U-shaped capillary 914 and into waste reservoir 515 (Figures 27 H through 27J). Preferably, sacrificial valves 922 are released to permit reagent buffer fluid flow.

The reagent buffer contains a chromogen or other developing agent for detection of specific binding in incubation chamber 910.

## 2. Resistive Heater and Temperature Sensing Components

Temperature control elements are provided to control the temperature of the platform. The invention provides heating elements, specifically resistive heating elements, and elements for detecting temperature at specific positions on the platform. Heating devices are preferably arrayed to control the temperature of the platform over a particular and defined area, and are provided having a steep temperature gradient with distance on the platform from the heater.

Certain resistors, including commercially-available resistive inks (available from Dupont) exhibit a positive temperature coefficient (PTC), *i.e.*, an increase in resistance with increasing temperature. Applying a fixed voltage across a PTC resistor screen-printed on a plastic substrate results in rapid heating, followed by self-regulation at an elevated temperature defined by the circuit design heat sink and ambient temperature. In such screen-printed resistors, connection to a power source is made by first printing parallel silver conductors followed by printing the PTC ink between the conductors as shown in Figure 29.

As shown in Figure 29, a resistive heating element comprises a conductive ink connected with electrical contacts for activation of the heater, and resistive inks applied between the conductive ink and in electrical contact therewith, wherein application of

a voltage (direct or alternating current) between the conductive inks results in current flow through the resistive inks and production of heat. There are two important types of resistive inks used in the resistive heating elements of this invention. The first is a standard polymer thick film ink, such as Dupont 7082 or Dupont 7102 ink. These inks produce a surface temperature that is not self-limiting, and the temperature resulting from the use of these inks is dependent primarily on the magnitude of the applied voltage. In contrast, the positive temperature coefficient (PTC) inks show increase resistivity with increasing voltage, so that surface temperature is self-limiting because the amount of heat-producing current goes down as the applied voltage goes up. PTC inks are characterized as having a particular temperature where this self-limiting property is first exhibited; at voltages that produce temperatures less than the critical temperature, the amount of heat is dependent on the magnitude of the applied voltage.

Resistive inks useful according to the invention include Dupont 7082, 7102, 7271, 7278 and 7285, and other equivalent commercially available polymer thick film ink and PTC inks.

Conductive inks useful according to the invention include Dupont 5028, 5025, Acheson 423SS, 426SS and SS24890, and other equivalent commercially available conductive inks.

Additional components of the dielectric layer that serves to insulate the electrical circuit. Dielectric layers advantageously comprise dielectric inks such as Dupont 5018A. Insulation can also be achieved using pressure sensitive transfer adhesive such as 7952MP (3M Co.), or a pressure sensitive transfer adhesive deposited onto a polyester carrier layer such as 7953MP (3M Co.) or thermoplastic bonding films such as 3M 406, 560 or 615.

Resistive heaters of the invention are advantageously used to incubate fluids at a stable temperature and for melting sacrificial valves as described below, and also for thermal cyclic.

Resistive and conductive inks are preferably screen-printed using methods and techniques well known in the art. See Gilleo, 1995, *Polymer Thick Film* (Van Nostrand Reinhold). Inks are typically screen printed to a thickness of about 10 microns; however, repetitive screen printing of resistive inks can be used to deposit thicker layers

having reduced resistances. Both conductive and resistive inks are heat cured, typically at between 110°C and 120°C for about 10 minutes. The outline of this printing process is shown in Figure 30. Importantly, each of the layers must be correctly registered with one another for resistive heating to be provided. Heaters can be screen printed to any  
5 required size; a minimum area for a screen-printed heater has been determined to be about 0.25mm<sup>2</sup> (0.5mm x 0.5mm).

The ability to tailor the resistance (and hence the temperature profile) of the resistive heaters using choice of ink formulation and reprinting of heater circuits provides control of the final electrical and thermal properties of the resistive heating  
10 elements of the invention. The resistance can also be controlled through connection of series and parallel configurations of resistive elements. For example, the particular circuits shown in Figure 31 allow for many parallel resistive elements per unit area; other configurations can be chosen for other applications.

### 15 3. Sacrificial Valves

The ability to specifically generate heat at a particular location on a microsystems platform of the invention also enables the use of sacrificial valves that can be released or dissolved using heat. For the purposes of this invention, the term "sacrificial valve" is intended to encompass materials comprising waxes, plastics, and other material that  
20 can form a solid or semi-solid fluid-tight obstruction in a microchannel, capillary, chamber, reservoir or other microfluidics component of the platforms of the invention, and that can be melted or deformed to remove the obstruction with the application of heat. Sacrificial valves are preferably made of a fungible material that can be removed from the fluid flow path. In preferred embodiments, said sacrificial valves are wax  
25 valves and are removed from the fluid flow path by heating, using any of a variety of heating means including infrared illumination and most preferably by activation of resistive heating elements on or embedded in the platform surface as described herein. For the purposes of this invention, the term "wax" is intended to encompass any solid, semi-solid or viscous liquid hydrocarbon, or a plastic. Examples include monodisperse  
30 hydrocarbons such as eicosane, tetracosane and octasone, and polydisperse hydrocarbons such as paraffin. In the use of wax sacrificial valves, application of a temperature higher

than the melting temperature of the wax melts the valve and removes the occlusion from the microchannel, capillary or other fluidic component of the microsystems platforms of the invention. Particularly when the sacrificial valve is melted on a rotating microsystems platform of the invention, the melted wax to flow through the microchannel, capillary or other fluidic component of the microsystems platforms of the invention and away from the original site of the valve.

One drawback, however, is the possibility that the wax will recrystallize as it flows away from the original valve site, and concomitantly, away from the localized heat source. Recrystallization results in re-occlusion of the microchannel, capillary or other fluidic component of the microsystems platforms of the invention, potentially and most likely at a site other than the site of a localized heat source, and therefore likely to foul fluid movement on the disc. One solution for this problem is the inclusion in the sacrificial wax valves of the invention of a wax recrystallization chamber positioned "downstream" from the position of the wax valve. Preferably, the wax recrystallization chamber is fluidly connected with the microchannel, capillary or other fluidic component of the microsystems platforms of the invention that was occluded by the wax sacrificial valve. Typically, the wax recrystallization chamber is a widening of the microchannel, capillary or other fluidic component of the microsystems platforms of the invention so that recrystallized wax can harden on the walls of the microchannel, capillary or other fluidic component of the microsystems platforms of the invention with enough distance between said walls that the recrystallized wax does not re-occlude the microchannel, capillary or other fluidic component of the microsystems platforms of the invention. Preferably, the heating element, most preferably the resistive heating element of the invention, extends past the site of the wax valve and overlaps at least a portion of the wax recrystallization chamber, thereby retarding the propensity of the wax valve to recrystallize.

It is also recognized that this propensity of wax valves to recrystallize can be exploited to create a wax valve at a particular location in a microchannel, capillary or other fluidic component of the microsystems platforms of the invention. In this embodiment, a particular location can be kept below a threshold temperature by failing to apply heat at that location, and a wax valve material can be mobilized from a storage

area on a platform by heating and then allowed to flow under centripetal acceleration to a particularly "cold" site where a wax valve is desired. An advantage of wax valves in this regard is that the proper positioning and activation of resistive heater elements enables flexibility in choosing when and whether a particular microchannel, capillary or other fluidic component of the microsystems platforms of the invention is to be occluded by a wax sacrificial valve.

In particularly preferred embodiments, the sacrificial valves of the invention comprise a cross-linked polymer that displays thermal recover, most preferably a cross-linked, prestressed, semicrystalline polymer; an example of a commercially available embodiment of such a polymer is heat recoverable tubing (#FP301H, 3M Co., Minneapolis, MN). Using these materials, at a temperature less than the "melting" temperature ( $T_m$ ), the polymer occludes a microchannel, capillary or other fluidic component of the microsystems platforms of the invention. At a temperature greater than  $T_m$ , however, the polymer reverts to its pre-stressed dimensions by shrinking. Such shrinking is accompanied by release of the occlusion from the microchannel, capillary or other fluidic component of the microsystems platforms of the invention. Such embodiments are particularly preferred because the polymer remains *in situ* and does not recrystallize or otherwise re-occlude the microchannel, capillary or other fluidic component of the microsystems platforms of the invention. Also, such embodiments do not require the more extensive manipulation in preparing the platforms of the invention that wax valves require.

In another embodiment, the sacrificial valves of the invention comprise a thin polymeric layer or barrier dividing two liquid-containing microchannel, capillary or other fluidic component of the microsystems platforms of the invention, that can burst when sufficient temperature and/or pressure is applied.

Another embodiment of the sacrificial valves of the invention are provided wherein a screen-printed resistive heater element is itself a valve. In this embodiment, the resistive heater element is screen-printed on a substrate such as polyester that divides two liquid-containing microchannel, capillary or other fluidic component of the microsystems platforms of the invention. In these embodiments, localized application of heat using a resistive heating element is used to melt the substrate dividing the liquid-



containing microchannel, capillary or other fluidic component of the microsystems platforms of the invention. Preferably, in this embodiment the two liquid-containing microchannel, capillary or other fluidic component of the microsystems platforms of the invention are positioned in adjacent layers through the vertical thickness of the platform.

5           As described above, the screen-printed resistive heater elements of this invention provide localized application of heat to a microsystems platform. The degree of localization achieved using these resistive heating elements is sufficient to provide for the placement of two adjacent sacrificial valves separated by a distance of 0.15cm.

10

#### 4.       **Electrical Connection through a Slip Ring Rotor**

          The invention also provides a specialized rotor spindle for making electrical connection through a spindle to a rotating structure. An example of the specialized spindle structure of the invention is shown in Figure 1. The spindle is provided with a  
15       series of electrically conductive rings, each of which are electrically isolated from the others. Each conductive ring is led to contacts that impinge upon circuits resident on the rotating structure. Electrical power or data signals are delivered to and from the apparatus by means of conductive brushes that contact the conductive rings. Signals may be conducted while the apparatus is rotating or when stationary.

20

          Rotating structures such as centrifugal rotors and the microsystems platforms of this invention containing internal channels and reservoirs are used to control fluid movement. Such disks and rotors are known in the art as centrifugal analyzers and have been used to separation chemical and biochemical species as well as enabling the synthesis of chemical and biochemicals.

25

          However, a limitation of conventional centrifugal analyzers is that those analytical or synthetic procedures that required electrical input on the rotating structure itself were not enabled. Instead, mechanical and non-mechanical processes were used to regulate fluid movement in centrifuge rotors and other centripetally-motivated devices. Mechanical processes included valve actuation and pumping liquid toward the  
30       center of rotation; non-mechanical processes included heating, cooling, electrophoresis, and sensing through combinations of optical, electrical, chemical and biological means.

The mechanical and non-mechanical processes known in the prior art were not capable of precisely regulating fluid movement when compared with equivalent, non-rotating devices that used electrical power.

Rotating structures permit precise control of fluid movement. Conventional centrifugal fluid control is restricted to outward flow in a radial direction that is primarily regulated through liquid placement (such as volume, positional radius, and liquid height), channel geometry (including considerations of channel radius) and rotational rate.

Temperature control is achieved over the entire rotor, by refrigerating the centrifugal compartment, for example. However, many chemical and biological processes require precisely controlled elevated temperature for optimal performance; for example, enzymatic reactions such as those used in immunoassays often have temperature optima of about 37°C. *In vitro* amplification reactions (such as polymerase chain reaction) require even more elevated temperatures, such as cycling between 70°C and 95°C.

A rotating structure presents unique difficulties in terms of thermal control, particularly heating. Conventional options included placement of the centrifuge rotor inside of an oven, subjecting one area to infrared radiation, and clamping the device between thermally controlled platens. Each of these approaches present difficulties. Ovens have unpredictable thermal gradients that prevent tight control of temperature in the interior of a rotating structure. Infrared presents similar difficulties, while clamping precludes spinning while heating unless the entire heating apparatus is also spun. All of these approaches suffer from an inability to directly measure the temperature inside the rotating structure. These reasons and other have prevented full integration of centripetally-motivated separation with analytical procedures in a single centrifugal rotor; typically, separations and analysis are performed separately in the prior art.

This invention provides a solution to this problem, specifically by enabling the transmission of electrical signals between a rotating structure such as a centrifuge rotor or microsystem platform of this invention and a fixed position electrical source. Electrical devices that can be advantageously controlled on a spinning rotor include temperature regulation devices, sensors, electrophoretic devices, integrated circuits and

mechanical valves. These structures in turn permits a wider array of chemical processes to be performed on a disk than those permitted with current technology.

The invention provides a specialized rotor spindle for transmitting electrical signals between a rotating centrifuge rotor and a stationary electrical source as follows; the structure of the spindle will be best appreciated with reference to Figure 1. Into an electrically non-conductive plate is embedded a plurality of electrically conductive posts. The non-conductive plate is made of any insulating, electrically non-conductive material, including most preferably hardened plastics, rubber, WHAT ELSE??, and other electrical insulators and non-conductors. The electrically conductive posts are made from any electrically conducting metal, including copper, aluminum, WHAT ELSE???, and are preferably spring-loaded from the bottom of the non-conductive plate. A mechanical spindle is positioned on the side of the non-conductive plate opposite to the side of the plate containing the conductive posts. This plate is referred to as a spindle plate. A number of conductive disks equal to the number of conductive posts are layered on the spindle plate. The electrical contacts in the conductive plate are arranged so that each conductive plate is in electrical contact with a single conductive post. For the purposes of this invention, the term "in electrical contact" is intended to mean that an electric current can be produced through the electrical contacts at voltages (direct or alternative current) that may be effectively achieved in a centrifugal apparatus as described. Each conductive disk is similarly electrically isolated from other disks with interspersed sheets or disks of nonconducting material, preferably the same non-conductive material used to make the non-conductive plate. The last layer positioned farthest way from the spindle plate is a nonconductive layer and is arranged so that the tips of the conductive posts are at the surface or emerge from this plate. This final plate containing the ends of the conductive posts is referred to as the contact plate. An assembled unit, consisting of baseplate, posts, conductive element within the stack, is referred to as a signal channel. Typically one channel will be reserved as a common or ground channel.

The contact stack is held within a chassis that permits a series of electrically conductive brushes to impinge upon each of the conductive disks of the contact stack. These brushes are arranged such that an electric signal can be propagated from one brush

to one contact on the contact plate independently of electrical signals conducted from any other brush. The spindle is held in a spring-loaded bearing assembly such that the contact stack can rotate freely and present resistive pressure when pressed from below. The assembled unit is referred to as an electronic spindle.

5           In the use of the electronic rotor of the invention, a centrifugal rotor or microsystems platform disk of this invention containing electrical contacts is aligned with the contacts on the contact plate of the electronic spindle in such a fashion that the electrical contact on the rotor are in electrical contact with the conductive posts on the contact plate of the electronic spindle. An electric signal can be propagated from the  
10 brushes of the electronic spindle to the contacts on the disk using the electronic spindle, most importantly when the rotor is spinning. Contact between the contact plate and the rotor is maintained by positive pressure provided by the spring-loaded bearing assembly and conductive posts. Electrical devices resident on the disk can then be monitored or controlled by signals through the electronic rotor. In a preferred embodiment, the rotor  
15 and the spindle contain complementary mechanical components that precisely align the rotor on the spindle to ensure proper electrical contact between the contacts on the rotor and the conductive posts on the spindle.

          In a preferred embodiment thermal control structures resident on a rotating disk may be controlled through the electronic spindle. A resistive heating element prepared  
20 in a rotor as described herein is prepared having electrical contact leads positioned to be brought into electrical contact with one signal channel and the common channel of the electronic spindle. A thermistor element as described herein is placed on the disk in close proximity to the resistive heating element to enable thermistor response to be proportional to the temperature of the resistive heating element. The temperature of the  
25 heating element will be a function of the voltage and current applied to the disk through the electronic rotor and the speed at which the disk is being rotated (which will promote convective cooling). A thermal profile may be accurately monitored through the thermistor response and temperature controlled through adjustment of heating voltage and disk speed using the electronic spindle of the invention.

30           In another preferred embodiment, the polymerase chain reaction ("PCR") can be

carried out on a rotating disk through thermal control using the electronic spindle. In this embodiment, a reaction chamber on a rotating disk is provided with the reagents necessary for PCR to provide a reaction mixture (*see* Saiki *et al.*, 1985, *Science* \_\_: \_\_-\_\_). This reaction chamber is in close proximity to a heater and thermistor as described  
5 herein to enable thermistor output to be proportional to the temperature of the reaction mixture. The reaction chamber is subjected to a cycling temperature profile sufficient to permit amplification of the template. Since the rate and precision at which a temperature change can be accomplished is a determining factor in the success of PCR amplification, control of voltage and speed of rotation as enabled using the electronic  
10 spindle of the invention permits PCR to be performed on a rotating platform.

In yet another preferred embodiment, other enzyme-requiring assays (such as enzyme linked immunosorbant assay ("ELISA")) can be performed and optimized on a rotating platform through thermal control using the electronic spindle of the invention. As those skilled in the art perform it, ELISA assays consist of antibody/antigen binding  
15 interactions followed by the conversion of a colorimetric or radioactive substrate into a detectable product. Detection is carried out through electrochemical or optical processes, depending the nature of the detectable product. Both antibody/antigen binding and enzymatic reactions have optimal temperatures (typically 37°C) that can be achieved on a rotating platform using the resistive heater/ thermistor pair described  
20 above using the electronic spindle.

Electromechanical valves known in the prior art can also be incorporated in a rotating platform of centrifuge rotor using the electronic spindle of the invention. Mechanical, electrolytic or thermal valves on a rotating disk have been described (for example, in co-owned and co-pending U.S. Serial No. 08/761,063). By judicious  
25 activation of such valves the fractionation of complex mixtures can be carried out on a rotating platform. In preferred embodiments, complex biological mixtures such as milk or blood are subjected to centrifugal force to effect separation of constituent parts. Electromechanical, electrolytic or thermal valves can be opened, for example, to withdraw a fractionated supernatant away from a sedimented layer and into an adjacent  
30 chamber. Sample fractionation can be accomplished using repetitive sedimentation at different rotational rates, with appropriate valve activation used to partition portions of

the fractionated to separate reservoirs or other compartments on the rotor. Upon fractionation the constituents may then be subjected to other processes such as PCR, immunoassay or electrophoresis.

Another application of the electronic spindle of the invention is to activate  
5 sensors on the rotating platform. In a preferred embodiment, sensors for detecting pH, are controlled and monitored through the electronic spindle.

The following Examples are intended to further illustrate certain preferred embodiments of the invention and are not limiting in nature.

10

### EXAMPLE 1

#### Antibiotic Assay Disk

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A microsystems platform provided by the invention and specifically designed for performing antibiotic assays is illustrated in Figures 1 and 2. Disk embodiments of the platforms of the invention were fashioned from machined acrylic and injection-molded polycarbonate. The overall disc dimensions include an outer radius of about 6cm and an inner radius of about 0.75cm, wherein the disk was mounted on the spindle of a rotary device. The thickness of the disc ranged from about 0.9mm to about 1.5mm.

20

The components of the antibiotic array were prepared as follows. An entry port **201** having a depth in the platform surface from about 0.75mm and lateral dimensions of from about 0.2cm to about 2cm were constructed on the platform, and designed to accommodate a volume of about 60 $\mu$ L. This entry port was fluidly connected with an array of eight metering capillaries **202** having a square cross-sectional diameter of about 0.5mm and proximal ends rounded with respect to entry port **201**; the length of this metering capillary array was sufficient to contain a total volume of about 20 $\mu$ L. The entry port was also constructed to be fluidly connected with an overflow capillary **203**  
25 having a cross-sectional diameter of from about 0.02mm to about 0.75mm and proximal ends rounded with respect to entry port **201**. The overflow capillary was fluidly connected with an overflow chamber **205** having a depth in the platform surface of about 0.75mm, greater than the depth of the overflow capillary **203**. Metering capillary **202**  
30 was fluidly connected to fluid chamber **204** having a depth in the platform surface of about 0.63mm and greater than the depth of the metering capillary **202**. Each of the

overflow and fluid chambers was also connected with air ports or air channels, such as 211, that have dimensions of about 0.25mm deep and permitted venting of air displaced by fluid movement on the platform. A capillary junction 212 that is about 0.75mm deep is present in the air channel to prevent fluid flow into the air channel.

5           Entry port 201 was positioned on the platform from about 2cm from the center of rotation. Metering capillary 202 extended about 1cm from entry port 201. The extent of the length of overflow capillary 203 was at least about 20% greater than the extent of the length of metering capillary 202. The position of fluid chamber 204 was from about 3.2cm from the center of rotation, and the position of overflow chamber 205 was thus  
10          from about 5cm from the axis of rotation.

          The fluid chamber 204 acted as a capillary barrier that prevents fluid flow from metering capillary 202 at a first, non-zero rotational speed  $f_1$  sufficient to permit fluid flow comprising overflow from the entry port 201 through overflow capillary 203 and into overflow chamber 205. This capillary boundary was constructed to be overcome  
15          at a second rotational speed  $f_2$  (where  $f_2 > f_1$ ). Fluid chamber 204 was fluidly connected to capillary 206 that was 0.25mm deep and had a cross-sectional diameter of about 0.5mm and was connected to holding chamber 207. Holding chamber 207 had a depth in the platform surface of 0.75mm, greater than the depth of capillary 206. Filling of fluid chamber 204 is accompanied by fluid flow through capillary 206 into holding  
20          chamber 207. Holding chamber 207 was fluidly connected by way of capillary 208, having a square cross-sectional diameter of about 0.25mm and was connected with read chamber 210, having a depth in the platform surface of about 0.75mm, greater than the depth of capillary 208. In certain embodiments, a sacrificial valve 213 was placed as shown in the channel 209.

25           As illustrated in Figures 3A through 3J, in the use of this platform an imprecise volume (ranging from 20-60 $\mu$ L of fluid) of a fluid was applied to the entry port 201 (Figure 3A). In embodiments of the platform comprising air displacement channels, the fluid wicked into air channel 211 and was stopped by capillary junction 212. Fluid also wicked into metering capillary 202 and overflow capillary 203. Fluid flowed through  
30          the metering capillary 202 and overflow capillary 203 at no rotational speed until the fluid reached capillary junctions at the junction between metering capillary 202 and fluid

chamber 204 and overflow capillary 203 and overflow chamber 205 (Figures 3B and 3C). Metering capillary 202 was constructed to define a precise volume from about 20-60 $\mu$ L of fluid between entry port 201 and the capillary junction at fluid chamber 204, which was designed to be at least the amount of the fluid placed by the user in entry port 201.

After sample loading by a user and filling of metering capillary 202 and overflow capillary 203 at no rotational speed, the platform was spun at a first rotational speed  $f_1$ , ranging from of 175rpm, which was sufficient for this microfluidics array having an entry port 201 with a depth of 0.6mm, metering capillary 202 with dimensions of 0.5mm x 0.5mm in cross-section and a length of 2.2-3.8cm from the center of rotation and an overflow capillary 203 with dimensions of 0.5mm x 0.5mm in cross-section and a length of 5.4cm from the center of.

Due to the greater distance of the end of overflow capillary 203 from the center of rotation than the end of metering capillary 202, fluid flowed through overflow capillary 203 into overflow chamber 205. The platform was spun until all excess fluid is evacuated from entry port 201 and into overflow chamber 205, except the fluid contained in metering capillary 202 (Figure 3D).

At a second rotational speed  $f_2$  of 360rpm, the precise amount of fluid contained in metering capillary 202 was delivered into fluid chamber 204 (Figures 3E through 3H). Fluid movement into fluid chamber 204 was accompanied by filling of capillary 206 and holding chamber 207.

In embodiments comprising a sacrificial valve 213 in-line with capillary 208 at position 209 shown in Figure 2, release of the sacrificial valve resulted in fluid flow into read chamber 210. In said embodiments, fluid flow is achieved at rotational speed  $f_2$  with removal of the sacrificial valve. In embodiments of the platforms of the invention comprising antibiotic arrays as described herein and not containing a sacrificial valve at position 209, capillary 208 preferably filled along with filling of holding chamber 207 until the fluid reached capillary junction 209 at the junction between capillary 208 and read chamber 210; in such embodiments, the capillary junction had a depth of about 0.75mm. At a third rotational speed  $f_3$  of about 520rpm, the fluid contained in holding chamber 207 was delivered into read chamber 210



(Figures 3I and 3J).

This embodiment of the microfluidics platforms of the invention was designed to use the carboxypeptidase inhibition assay described above for detecting beta-lactam antibiotics. The extent of chromogen production was detected in the read chamber, and related to the presence of antibiotics in the sample by comparison with samples tested in the absence of antibiotic. The amount of antibiotic in a test sample was determined using this platform of the invention.

## EXAMPLE 2

### Two-Step Assay Disk: Alternative Embodiment

In an alternative embodiment, a two-step assay disk of the invention was provided as shown in Figures 4 and 5. Disk embodiments of the platforms of the invention were fashioned from machined acrylic. The overall disc dimensions included an outer radius of about 6cm and an inner radius of about 0.75cm, wherein the disk was mounted on the spindle of a rotary device. The thickness of the disc ranged from about 0.9mm to about 1.5mm. The working fluid volume for reaction with reagents was about 20 $\mu$ L.

The components of this two-step assay array were prepared as follows. An entry port 301 having a depth in the platform surface of about 0.75mm and lateral dimensions of from about 0.2cm to about 2cm was constructed on the platform, and designed to accommodate a volume of about 60 $\mu$ L. This entry port was fluidly connected with a multiplicity of entry capillaries 302 having a square cross-sectional diameter of about 0.5mm, having a depth of 0.5mm and proximal ends rounded with respect to entry port 301; the length of this entry capillary array was sufficient to contain a total volume of about 20 $\mu$ L. The entry capillaries 302 were fluidly connected to fluid chamber 303 having a depth in the platform surface of about 0.6mm, wherein the depth was greater than the depth of the entry capillary 302. Each of the fluid chambers of this aspect of the invention was also connected with air ports or air channels, such as 311, that have dimensions of about 0.25mm deep and permitted venting of air displaced by fluid movement on the platform. A capillary junction 312 that was about 0.75mm deep was present in the air channel to prevent fluid flow into the air channel.

The fluid chamber 303 was also constructed to be fluidly connected with an overflow capillary 304 having a cross-sectional diameter of from about 0.02mm to about 0.75mm and proximal ends rounded with respect to fluid chamber 304. The overflow capillary was fluidly connected with an overflow chamber 306 having a depth in the platform surface of about 0.75mm, greater than the depth of the overflow capillary 304.

Entry port 301 was positioned on the platform about 1cm from the center of rotation. Entry capillaries 302 extends about 2cm from entry port 301. The position of a first fluid chamber 303 was from about 3cm from the center of rotation.

The first fluid chamber 303 acted as a capillary barrier that prevented fluid flow from entry capillary 302 at zero rotational speed. Movement of fluid from entry port 301 through entry capillaries 302 and into the first fluid chamber 303 was achieved by rotation at a first, non-zero rotational speed  $f_1$ . Displacement of fluid into the first fluid chamber 303 was accompanied by fluid filling of channel 305 that was fluidly connected with the first fluid chamber 303 and was positioned at the most radially distal point of the first fluid chamber. Channel 305 was fluidly connected with a second fluid chamber 307 and formed a capillary boundary between channel 305 and chamber 307. This capillary boundary was constructed to be overcome at second rotational speed  $f_2$  (where  $f_2 > f_1$ ). First fluid chamber 303 was also fluidly connected to overflow capillary 304 that was 0.25mm deep and had a cross-sectional diameter of about 0.5mm and that extended from about 1cm to about 5cm and was connected to overflow chamber 306. Overflow chamber 306 had a depth in the platform surface equal to that of overflow capillary 304, so that there was no capillary boundary between overflow capillary 304 and overflow chamber 306. Overflow capillary 304 was positioned in the first fluid chamber 303 at a point radially less distant from entry port 301 than channel 305, thereby defining a volume in the fluid chamber between the position of the overflow capillary 304 and the most radially distant extent of the said first fluid chamber.

Second fluid chamber 307 was further fluidly connected through channel 308 to a small pocket or capillary junction 309. Channel 308, having a cross-sectional diameter of about 0.25mm and that extended from about 0.2cm to about 20cm, was fluidly connected to a third fluid chamber 310, having a depth in the platform surface of

about 0.75mm, that was greater than the depth of capillary 308. Air recirculation channels 311 that had dimensions of about 0.25mm deep provided pathways for air displaced by fluid movement, while capillary junctions 312 that were about 0.75mm deep prevent fluid from entering the air channels. In some embodiments of the device a sacrificial valve 313 was placed as shown in Figure 5 in the channel 309. In certain  
5       embodiments, a valve 314 was placed in channel 305 to control fluid movement from the first fluid chamber 303 to the second fluid chamber 307.

As illustrated in Figures 6A through 6J, in the use of this platform an imprecise volume (ranging from 1-150 $\mu$ L of fluid) of a fluid was applied to the entry port 301  
10       (Figure 6A). Fluid wicked into entry capillary 302 and stopped at the capillary junction between entry capillary 302 and the first fluid chamber 303 (Figures 6B and 6C). Fluid flowed through the entry capillary B and into the first fluid chamber 303 at a first rotational speed  $f_1$  of 40rpm (Figures 6D and 6E). The fluid further entered capillary channel 305, stopping at the capillary junction with the second fluid chamber 307. As  
15       rotation continued, the fluid continued to fill the first fluid chamber 303, overflow capillary 304 fills (Figure 6F), and excess fluid filled overflow chamber 306 until the level of fluid in the first fluid chamber 303 fell below the position of overflow capillary 304 (Figure 6G).

At a second rotational speed  $f_2$  of 280rpm, the capillary junction between channel  
20       305 and the second fluid chamber 307 was overcome, and fluid remaining in the first fluid chamber 303 was delivered into the second fluid chamber 307 (Figures 6H and 6I).

In an alternative embodiment, a sacrificial valve 314 was placed at the junction of channel 305 and the second fluid chamber 308, which sacrificial valve was released to permit fluid flow through channel 305 and into the second fluid chamber 308. In such  
25       embodiments, fluid flow can be achieved at either  $f_1$  or  $f_2$  rotational velocity.

In embodiments comprising a sacrificial valve 313 in-line with capillary 308 at position 309 shown in Figure 5, release of the sacrificial valve results in fluid flow into the third fluid chamber 310. In said embodiments, fluid flow was achieved at rotational speed  $f_2$  with removal of the sacrificial valve.

30       In embodiments of the platforms of the invention comprising two-step assay arrays as described herein and not containing a sacrificial valve at position 310, capillary

309 filled along with filling of the second chamber 308 until the fluid reached capillary junction 309 at the junction between capillary 308 and the third fluid chamber 310; in such embodiments, the capillary junction has a depth of about 0.75mm. At a third rotational speed  $f_3$  of about \_\_\_\_ rpm, the fluid contained in the second chamber 308 was delivered into the third fluid chamber 310 (Figures 6H through 6K).

### EXAMPLE 3

#### Blood Separation Array

A microsystems platform provided by the invention and specifically designed for separating vertebrate blood cells and components is illustrated in Figures 7 through 9.

The components of the blood separation array are shown in greater detail in Figure 8. It will be understood by a comparison of Figures 7 and 8 that the center of the platform 11 is at the top of Figure 8, and the edge or lateral extent of the platform is at the bottom of Figure 8, illustrated by a curved line. Rotation of the blood separation array on platform disks of the invention can be in either direction, although rotation in a consistent, particular direction is preferred. Disk embodiments of the platforms of the invention were fashioned from machined acrylic. The overall disc dimensions included an outer radius of about 6cm and an inner radius of about 0.75cm, wherein the disk is mounted on the spindle of a rotary device. The thickness of the disc ranged from about 0.9mm to about 1.5mm. The working fluid volume for reaction with reagents was about 15 $\mu$ L.

The components of the blood separation array were as follows. An entry port 401 having a depth in the platform surface of about 0.5mm and lateral dimensions of about 0.5cm was constructed on the platform, and designed to accommodate a volume of about 20 $\mu$ L. This entry port was fluidly connected to an entry capillary 402, having a cross-sectional diameter of about 0.5mm and having a depth of 0.5mm; the length of this entry capillary was sufficient to contain a total volume of about 20 $\mu$ L. Entry capillary 402 was further fluidly connected to a separation column 403 having a cross-sectional diameter of about 1.25mm, a depth of about 0.75mm, and a length of this separation column was sufficient to contain a total volume of about 15 $\mu$ L. This separation column was also fluidly connected with a passage 411 to overflow chamber

404. Passage 411 had a cross-sectional diameter of about 1mm, a depth of from about 0.5mm, and a length of 2mm. Overflow chamber 404 has a depth of about 0.5mm.

A small capillary exit 406 was also fluidly connected with separation chamber 403, having a cross-sectional diameter of about 0.125mm, a depth of about 0.125mm, and a length of about 0.75mm. This capillary was arranged to traverse a direction radially more proximal to the axis of rotation than the insertion point with separation column 403. This small capillary 406 terminated in a capillary junction 407 that was fluidly connected with capillary 408, extending in a radial direction to decant chamber 405. A sacrificial valve 413 is positioned in capillary 406 at the juncture with capillary junction 407. Capillary 408 had a cross-sectional diameter of about 0.25mm, a depth of about 0.25mm, and a length of about 3.5mm. This capillary was arranged in a radially outward direction between capillary junction 407 and decant chamber 405. Passage 411 was positioned on separation column 403 to be significantly more proximal to the axis of rotation than the insertion point of small capillary 406.

Air displacement channels 409 that have dimensions of about 0.25mm deep permitted venting of air displaced by fluid movement on the platform. Capillary junctions 410 that were about 0.75mm deep were present in the air channels to prevent fluid flow into the air channels.

As illustrated in Figures 9A through 9H, in the use of this platform an imprecise volume (about 25 $\mu$ L) of blood was applied to the entry port 401 (Figure 9A). Blood entered the entry capillary 402 by capillary action, and stopped at the capillary junction between entry capillary 402 and the separation chamber 403 (Figures 9B and 9C).

At a first rotational speed  $f_1$  of 150rpm, blood flowed from the entry capillary 402 into separation chamber 403 (Figure 9D). Blood continued to fill separation column 403 until blood reached the position of passage 411, whereupon excess blood flowed through passage 411 and into overflow chamber 404 (Figures 4E and 4F). Advantageously, small channel 406 had dimensions that prevent wicking of blood into the channel as blood flows past the insertion point of small channel 406 into separation column 403.

As shown in Figure 9F, after sufficient time of rotation at the first non-zero rotational speed  $f_1$ , the excess blood has been transferred into overflow chamber 404 and

the separation column 403 was filled with blood to the position of passage 411. Rotation at a second rotational speed  $f_2$  of 1300rpm, blood components were separated into red blood cell, white blood cell (*i.e.*, "buffy coat"), and plasma fractions (Figure 9G). Advantageous dimensions of small capillary 406 permitted fluid flow of the plasma fraction through capillary 406 that was stopped at capillary junction 407. Fluid flow of plasma into decant chamber 405 resulted from fluid flow overcoming the capillary barrier 407 by rotation at a third rotational speed  $f_3$  of about 1420rpm (Figure 9H).

#### EXAMPLE 4

##### Blood Separation Array: Alternative Embodiment

In an alternative embodiment, a blood separation disk of the invention is provided as shown in Figures 10 and 11. It will be understood that, as in Example 1, in Figure 10, the arrangement of one separation array 15 on a disk 11 is shown; a multiplicity of such arrays can be advantageously arranged on a microsystems platform, most preferably a disk, of the invention, to provide a multi-use or multi-assay platform. Disk embodiments of the platforms of the invention are fashioned from machined acrylic. The overall disc dimensions include an outer radius of about 6cm and an inner radius of about 0.75cm, wherein the disk is mounted on the spindle of a rotary device. The thickness of the disc ranged from about 0.9mm to about 1.5mm. The working fluid volume for reaction with reagents is about 25 $\mu$ L.

The components of this separation array are as follows. An entry port 501 having a depth in the platform surface from about 0.75mm and lateral dimensions of from about 0.5cm is constructed on the platform, and designed to accommodate a volume of about 20 $\mu$ L. This entry port is fluidly connected with a first array of metering capillaries 502 and a second array of metering capillaries 503, wherein each of the capillaries has a cross-sectional diameter of about 0.5mm. The length of the second metering capillary array 503 is longer than that of the first metering capillary array 502. The first metering capillary array 502 is fluidly connected with a ballast chamber 507, having a depth in the platform surface of about 0.75mm and greater than the depth of the first metering capillary array 502, wherein the first metering capillary array 502 forms

a capillary junction between the array and the ballast chamber. The second capillary array 503 is fluidly connected with capillary junction 506.

5 The entry port is also constructed to be fluidly connected with an overflow capillary 504 having a cross-sectional diameter of about 0.5mm and proximal ends rounded with respect to entry port 501. The overflow capillary is fluidly connected with an overflow chamber 505 having a depth in the platform surface of about 0.75mm, greater than the depth of the overflow capillary 504. Each of the overflow and fluid chambers is also connected with air ports or air channels, such as 514, that have dimensions of about 0.25mm deep and permit venting of air displaced by fluid movement on the platform. Capillary junctions 516 that is about 0.75mm deep are present in the air channels to prevent fluid flow into the air channels.

10 Entry port 501 is positioned on the platform from 1cm to 20cm from the center of rotation. Metering capillary array 502 extends from entry port 501 from about 0.6cm. Metering capillary array 503 extends from entry port 501 from about 1.9cm. The extent of the length of metering capillary array 503 is about 20% longer than metering capillary array 502, and the extent of the length of overflow capillary 504 is at least about 20% greater than the extent of the length of either the first metering capillary array 502 or the second metering capillary array 503. The position of ballast chamber 507 is about 2.8cm from the center of rotation, the position of capillary junction 506 is about 3.8cm from the center of rotation, and the position of overflow chamber 505 is thus about 5cm from the axis of rotation.

20 The ballast chamber 507 acts as a capillary barrier that prevents fluid flow from the first metering capillary array 502 at a first, non-zero rotational speed  $f_1$  sufficient to permit fluid flow comprising excess blood overflow from the entry port 501 through overflow capillary 504 and into overflow chamber 505. Capillary junction 506 is a capillary barrier that prevents fluid flow from the second metering capillary array 503 at said first, non-zero rotational speed  $f_1$  sufficient to permit fluid flow comprising excess blood overflow from the entry port 501 through overflow capillary 504 and into overflow chamber 505. These capillary boundaries are constructed to be overcome at a second rotational speed  $f_2$  (where  $f_2 > f_1$ ).

30 Ballast chamber 507 is fluidly connected to capillary 510 that is 0.25mm deep

and has a cross-sectional diameter of about 0.65mm and that extends about 5cm and is connected to capillary junction 511. A sacrificial valve 518 is positioned at the exit of ballast chamber 507 at the juncture with capillary junction 511. Alternatively, capillary 510 is fluidly connected with a sacrificial valve 515. In said embodiments, fluid flow is achieved at rotational speed  $f_2$  with removal of the sacrificial valve. Sacrificial valve 515 or capillary junction 511 are further fluidly connected with channel 512 which is about 0.25mm deep and has a cross-sectional diameter of about 0.25mm and that extends about 3cm. Channel 512 is fluidly connected with separation chamber 509 at a point most distal from the axis of rotation.

Second metering capillary array 503 is fluidly connected with capillary junction 506, which is overcome at a rotational speed  $f_2 > f_1$ . Capillary junction 506 is further fluidly connected to channel 508, which is further fluidly connected to separation chamber 509. Channel 508 is about 0.25mm deep and has a cross-sectional diameter of about 0.25mm. Separation chamber 509 is about 0.75mm deep and has a cross-sectional diameter of about 5mm, and is positioned from about 40cm from the center of rotation.

Separation chamber 509 is fluidly connected with decant channel 517 at a point close to the chamber's most axis-proximal extent. Decant channel 517 is about 0.25mm deep and has a cross-sectional diameter of about 0.25mm and extends about 4.3cm to about 5cm. Decant channel 517 is fluidly connected with decant chamber 514, which is about 0.75mm deep and has a cross-sectional diameter of about 5mm, and is positioned from about 50cm to about 80cm from the center of rotation..

As illustrated in Figures 12A through 12J, in the use of this platform an imprecise volume (about 25 $\mu$ L of fluid) of blood is applied to the entry port 501 (Figure 12A). Blood enters the each of the metering capillary arrays 502 and 503 and stops at the capillary junction between metering capillary array 502 and ballast chamber 507 and between metering capillary 503 and capillary junction 506 (Figures 12B and 12C). Blood also enters and fills overflow capillary 504, stopping at the capillary junction with overflow chamber 505.

At a first rotational speed  $f_1$  of 45rpm, blood flows from the entry port 501 through overflow capillary 504 and into overflow chamber 505 (Figures 12D and 12E). At a second rotational speed  $f_2$  of 70rpm, the capillary junction between the first



metering capillary array 502 and ballast chamber 508 is overcome, and blood from the first metering capillary array fills ballast chamber 508 (Figure 12F). Similarly, at second rotational speed  $f_2$ , capillary junction 506 is overcome, and blood from second metering capillary array 503 enters separation chamber 509 (Figure 12F). Advantageously, the volume of blood in second metering capillary array 503 is insufficient to fill separation chamber 509 to the level of insertion of decant channel 517.

By rotation at a third rotational speed  $f_3$  of 1300rpm, blood components in separation chamber 509 are separated into red blood cell, white blood cell (*i.e.*, "buffy coat"), and plasma fractions (Figures 12G and 12H). Separation of blood components is not achieved in ballast chamber 507, due to its position on the platform, and the capillary junction 511 or sacrificial valve 515 are not overcome at third rotational speed  $f_3$ . Advantageously, the separated plasma does not extend to decant capillary 517.

Release of sacrificial valve 517, or rotation at a fourth rotational speed  $f_4$  of rpm, results in flow of blood from ballast chamber 507 through channel 512 and into separation chamber 509 at the "bottom" or most axis-distal extent of the separation chamber (Figure 12I). This results in filling of the separation chamber to a position equal to the insertion point of decant channel 517 (Figure 12J). Plasma flow through decant channel 517 and into decant chamber 514 in an amount equal to the amount of blood contained in ballast chamber 507. Decant channel 517 is advantageously provided with dimensions that retard passage of unfractionated blood, or plasma contaminated with greater than 0.1-1% of blood cells in whole blood.

## EXAMPLE 5

### Mixing Arrays

A microsystems platform provided by the invention and specifically designed for performing mixing of equal volumes of different liquid samples is illustrated in Figure 13. In the Figure, the arrangement of one assay array 15 on a disk 11 is shown; a multiplicity of such arrays can be advantageously arranged on a microsystems platform, most preferably a disk, of the invention, to provide a multi-use or multi-assay platform.

The components of the mixing array are shown in greater detail in Figure 14. Disk embodiments of the platforms of the invention were fashioned from machined

acrylic. The overall disc dimensions include an outer radius of about 6cm and an inner radius of about 0.75cm, wherein the disk is mounted on the spindle of a rotary device. The thickness of the disc ranged from about 0.9mm to about 1.5mm. The working fluid volume was about 50 $\mu$ L.

5           The components of the mixing array were prepared as follows. Entry ports 601 having a depth in the platform surface of about 0.5mm and lateral dimensions of about 1cm to about 5cm were constructed on the platform, and designed to accommodate a volume of about 5-50 $\mu$ L. Each entry port was fluidly connected with one of a paired array of metering capillaries 602 having a square cross-sectional diameter of about  
10           0.5mm and proximal ends rounded with respect to entry port 601; the length of each metering capillary array was sufficient to contain a total volume of about 25 $\mu$ L. Metering capillaries 602 were fluidly connected to a curved capillary barrier 603 having a depth in the platform surface of about 0.25mm that was greater than the depth of metering capillaries 602. The capillary barrier 603 and other fluid components of the  
15           mixing array were also connected with air channels 608, that have dimensions of about 0.25mm deep and permitted venting of air displaced by fluid movement on the platform. In addition, capillary junctions 609 that were about 0.75mm deep were present in the air channels to prevent fluid backflow into the air channel.

          Capillary barrier 603 was fluidly connected by a narrow capillary channel 604  
20           to mixing chamber 605, which was fluidly connected with channel 610, which was further connected with mixed fluid receiving chamber 606. Alternatively, capillary 604 comprises a sacrificial valve 612. Capillary channel 604 was about 0.25mm deep and had a cross-sectional diameter of about 0.25mm and that extends about 0.2cm. Mixing chamber 605 was about 0.75mm deep and had a cross-sectional diameter of about 2mm,  
25           and was positioned about 4cm from the center of rotation. Capillary channel 610 was about 0.25mm deep and had a cross-sectional diameter of about 0.25mm and that extended from about 0.2cm to about 30cm. Mixing chamber 605 was constructed such that the insertion point of capillary channel 604 and the insertion point of capillary channel 610 were offset at opposite ends of the mixing chamber. As a consequence,  
30           fluid flowing through capillary channel 604 was forced to encounter the opposite wall of mixing chamber 605 before fluid flow can proceed through capillary channel 610.

This results in the creation of turbulence in the mixed laminar fluid stream in capillary channel 604 caused by the conjoint flow of fluid from the first and second metering channels without appreciable mixing. The turbulence created by the structure of mixing chamber 605 was sufficient to disrupt laminar flow and cause fluid mixing in the chamber prior to continued fluid flow through capillary channel 610 and into mixed fluid receiving chamber 606. Mixed fluid receiving chamber 606 was about 0.75mm deep and had a cross-sectional diameter of about 5mm, and was positioned from about 1cm to about 30cm from the center of rotation.

As illustrated in Figures 15A through 15d, in the use of this platform an equal volume (ranging from 1-150 $\mu$ L of fluid) of each of the fluids to be mixed was applied to the entry ports 601 (Figure 15A). Fluid enters the each of the metering capillary arrays 602 and stops at capillary barrier 603.

At a first rotational speed  $f_1$  of 90rpm, the fluids from each capillary array flowed into and filled the capillary barrier 603 (Figure 15B). In embodiments comprising a sacrificial valve 612, the valve prevented fluid flow into channel 604. Upon release of sacrificial valve 612 or rotation at first rotational speed  $f_1$ , fluid flow proceeded from capillary junction 603 through channel 604 and into mixing chamber 605 (Figure 15C). Fluid flow within mixing chamber 605 was turbulent, in contrast to fluid flow through capillary barrier 603 or channel 604, which was primarily laminar, so that mixing occurs predominantly in mixing chamber 605. Fluid flow proceed through channel 610 and the mixed fluid solution was displaced into mixed fluid receiving chamber 606 (Figure 15D).

## EXAMPLE 6

### Mixing Arrays: First Alternatives

An additional embodiment of the microsystems platform provided by the invention and specifically designed for performing mixing of equal volumes (Figures 17 through 18) or unequal volumes (Figures 19 through 21) of different liquid samples. In these Figures, the arrangement of one assay array 17 on a disk 11 is shown; a multiplicity of such arrays can be advantageously arranged on a microsystems platform, most preferably a disk, of the invention, to provide a multi-use or multi-assay platform.

The components of the mixing array are shown in greater detail in Figures 17 and

20. Disk embodiments of the platforms of the invention are fashioned from machined acrylic. The overall disc dimensions include an outer radius of about 6cm and an inner radius of about 0.75cm, wherein the disk is mounted on the spindle of a rotary device. The thickness of the disc ranged from about 0.9mm to about 1.5mm. The working fluid volume is about 40 $\mu$ L in each fluid reservoir.

The components of the mixing array are as follows. Mixing of equal volumes is illustrated in Figure 17 and for unequal volumes in Figure 20. (Components will be identified using the number from Figure 17; equivalent structures are shown in Figure 20 for unequal mixing and will be shown in parentheses). Fluid reservoirs 651 and 652 (701 and 702), each containing one of a pair of liquids to be mixed, are constructed on the platform, having a depth in the platform surface from about 0.75mm and lateral dimensions of 1cm, and wherein fluid reservoirs 651 and 652 are designed to accommodate equal volumes of fluid (about 50 $\mu$ L); (for unequal volumes fluid reservoir 701 is designed to accommodate a volume of about 45 $\mu$ L, and fluid reservoir 702 is designed to accommodate a volume of about 5 $\mu$ L, wherein the volume of fluid reservoir 702 is less than the volume of fluid reservoir 701.) In particular and in addition, the viscosity of the fluid in the fluid reservoirs may differ, so that mixing produces a mixed fluid of intermediate viscosity. Each fluid reservoir is fluidly connected with a capillary channel 653 and 654 (703 or 704) to capillary junction 655 (705). Each capillary channel is about 0.5mm deep and having a cross-sectional diameter of about 0.5mm and that extends about 5cm. Capillary junction 655 (705) has a depth in the platform surface of about 0.75mm that is greater than the depth of capillaries 652 and 653 (703 and 704). Alternatively, capillaries 652 and 653 (703 and 704) comprise a sacrificial valve 662 (712). Use of said sacrificial valves can be used in addition to or in place of capillary junction 655 (705).

The fluid components of the mixing array are also connected with air channels 660 (710), that have dimensions of about 0.25mm deep and permit venting of air displaced by fluid movement on the platform. In addition, capillary junctions 661 (711) that are about 0.75mm deep are present in the air channels to prevent fluid backflow into the air channel.

Capillary junction 655 (705) is fluidly connected by a narrow capillary channel 656 (706) to mixing chamber 657 (707), which is fluidly connected with channel 658 (708) which is further connected with mixed fluid receiving chamber 659 (709). Alternatively, capillary 656 (706) comprises a sacrificial valve 662 (712). Capillary channel 656 (706) is about 0.25mm deep and having a cross-sectional diameter of from about 0.5mm and that extends from about 0.2cm to about 30cm. Mixing chamber 657

(707) is about 0.25mm deep and having a cross-sectional diameter of from about 0.75mm, and is positioned from about 0.2cm to about 30cm from the center of rotation. Capillary channel 658 (708) is about 0.5mm deep and has a cross-sectional diameter of about 5mm and that extends from about 0.2cm to about 30cm. Capillary channel 656 (706) and capillary channel 658 (708) may be offset in their connection with the mixing chamber as previously described in Example 5, or the capillaries can be positioned in the mixing chamber at any convenient position, and Coriolis forces have be relied upon to facilitate mixing.

Capillary 658 (708) is fluidly connected with mixed fluid receiving chamber 659 (709). Mixed fluid receiving chamber 659 (709) is about 0.75mm deep and having a cross-sectional diameter of about 5mm from about, and is positioned from about 1cm to about 30cm from the center of rotation.

As illustrated in Figures 18E through 18E for mixing equal volumes, and in Figures 21A through 21E for mixing unequal volumes, in the use of this platform a volume of each of the fluids to be mixed is applied to the fluid reservoirs 651 and 652 (701 and 702) (Figures 18A and 21A). Fluid enters the each of the capillaries 653 and 654 (703 or 704) and stops at capillary junction 655 (705). Alternatively, the platforms of the invention are provided containing the fluids to be mixed already in fluid reservoirs 651 and 652 (701 and 702). In these embodiments, it is preferred that sacrificial valves 712 be provided in capillaries 653 and 654 (703 or 704), to prevent evaporation, wetting or leakage of fluid from the reservoirs prior to use.

At a first rotational speed  $f_1$  of 100rpm, the fluids from each capillary flows past capillary junction 655 (705) and through mixing chamber 657 (707) (Figures 18B and 18C and Figures 21B and 21C). In embodiments comprising a sacrificial valve 712, the valve prevents fluid flow into channels 653 and 654 (703 or 704). Upon release of sacrificial valve 712, fluid flow proceeds from capillary junction 655 (705) through channel 656 (706) and into mixing chamber 707 (Figure s 18C and 21C). Fluid flow within mixing chamber 657 (707) is turbulent, in contrast to fluid flow through capillary barrier 655 (705) or channel 656 (706), which is primarily laminar, so that mixing occurs predominantly in mixing chamber 657 (707). Fluid flow proceed through channel 658 (708) and the mixed fluid solution is displaced into mixed fluid receiving chamber 659 (709) (Figures 18D and 18 E and Figures 21D and 21E).

## EXAMPLE 7

### Mixing Arrays: Second Alternative

5 An additional embodiment of the microsystems platform provided by the invention and specifically designed for performing mixing of different volumes of liquid samples to form a gradient in the concentration of a species in which the two fluids differ; this embodiment is illustrated in Figure 22. In the Figure, the arrangement of one assay array 18 on a disk 11 is shown; a multiplicity of such arrays can be advantageously arranged on a microsystems platform, most preferably a disk, of the invention, to provide a multi-use or multi-assay platform.

10 The components of the mixing array are shown in greater detail in Figure 23. Disk embodiments of the platforms of the invention are fashioned from machined acrylic. The overall disc dimensions include an outer radius of about 6cm and an inner radius of about 0.75cm, wherein the disk is mounted on the spindle of a rotary device. The thickness of the disc ranged from about 0.9mm to about 1.5mm. The working fluid  
15 volume is about 40 $\mu$ L for each fluid reservoir.

The components of the mixing array are as follows. Fluid reservoirs 801 and 802, each containing one of a pair of liquids to be mixed, are constructed on the platform, having a depth in the platform surface from about 0.75mm and lateral dimensions of 1cm, and wherein fluid reservoir 801 is designed to accommodate a  
20 volume of about 40 $\mu$ L, and fluid reservoir 802 is designed to accommodate a volume of about 40 $\mu$ L, wherein the volume of fluid reservoir 802 is different than the volume of fluid reservoir 801. In particular and in addition, fluid reservoirs 801 and 802 are shaped so that the rate of fluid output in the two reservoirs differs between the reservoirs at a particular rotational speed, due to a change in the pressure "head" (related to the  
25 cross-sectional area of the fluid at each point in the reservoir), so that the proportion of fluid in the mixture from one of the reservoirs is at a maximum at the beginning of rotation and is at a minimum when the fluids from the reservoirs are completely mixed at the end of rotation, thus forming a gradient. Gradient should  
be approximately 40% of left reservoir and 60% of right reservoir fluid at beginning and  
30 reversed at end; aliquoting structure of other mixing array should provide a way to preserve this gradient.

Each fluid reservoir is fluidly connected with a capillary channel 803 or 804 to capillary junction 805. Each capillary channel is about 0.5mm deep and having a cross-sectional diameter of about 0.5mm and that extends about 5cm. Capillary junction 805 has a depth in the platform surface of about 0.75mm that is greater than the depth of capillaries 803 to 804. Alternatively, capillaries 803 or 804 comprise a sacrificial valve 812. Use of said sacrificial valves can be used in addition to or in place of capillary junction 805.

The fluid components of the mixing array are also connected with air channels 810, that have dimensions of about 0.25mm deep and permit venting of air displaced by fluid movement on the platform. In addition, capillary junctions 811 that are about 0.75mm deep are present in the air channels to prevent fluid backflow into the air channel.

Capillary junction 805 is fluidly connected by a narrow capillary channel 806 to mixing chamber 807, which is fluidly connected with channel 808, which is further connected with mixed fluid receiving chamber 809. Alternatively, capillary 806 comprises a sacrificial valve 812. Capillary channel 806 is about 0.5mm deep and having a cross-sectional diameter of from about 0.5mm and that extends from about 0.2cm to about 30cm. Mixing chamber 807 is about 0.75mm deep and having a cross-sectional diameter of from about 0.75mm, and is positioned from about 0.2cm to about 30cm from the center of rotation. Capillary channel 808 is about 0.5mm deep and has a cross-sectional diameter of about 5mm and that extends from about 0.2cm to about 30cm. Capillary channel 806 and capillary channel 808 may be offset in their connection with the mixing chamber as previously described in Example 5, or they can be positioned in the mixing chamber at any convenient position, and Coriolis forces have be relied upon to facilitate mixing.

Capillary 808 is fluidly connected with mixed fluid receiving chamber 809. Mixed fluid receiving chamber 809 is about 0.75mm deep and having a cross-sectional diameter of about 5mm from about, and is positioned from about 1cm to about 30cm from the center of rotation.

As illustrated in Figures 24A through 24E, in the use of this platform a volume (ranging from 5-45 $\mu$ L of fluid) of each of the fluids to be mixed is applied to the fluid

reservoirs 801 and 802 (Figure 24A). Fluid enters the each of the capillaries 803 and 804 and stops at capillary junction 805. Alternatively, the platforms of the invention are provided containing the fluids to be mixed already in fluid reservoirs 801 and 802. In these embodiments, it is preferred that sacrificial valves 812 be provided in capillaries 803 and 804, to prevent evaporation, wetting or leakage of fluid from the reservoirs prior to use.

At a first rotational speed  $f_1$  of 100rpm, the fluids from each capillary flows past capillary junction 805 and through mixing chamber 807 (Figures 24B and 24C). In embodiments comprising a sacrificial valve 812, the valve prevents fluid flow into channels 803 and 804. Upon release of sacrificial valve 812, fluid flow proceeds from capillary junction 805 through channel 806 and into mixing chamber 807 (Figure 24D). Fluid flow within mixing chamber 807 is turbulent, in contrast to fluid flow through capillary barrier 805 or channel 806, which is primarily laminar, so that mixing occurs predominantly in mixing chamber 807. Fluid flow proceed through channel 808 and the mixed fluid solution is displaced into mixed fluid receiving chamber 809 (Figures 24D and 24E).

## EXAMPLE 8

### Immunoassays

A microsystems platform provided by the invention and specifically designed for performing immunoassay is illustrated in Figure 25. In the Figure, the arrangement of one assay array 19 on a disk 11 is shown; a multiplicity of such arrays can be advantageously arranged on a microsystems platform, most preferably a disk, of the invention, to provide a multi-use or multi-assay platform.

The components of the mixing array are shown in greater detail in Figure 26. Disk embodiments of the platforms of the invention were fashioned from machined acrylic. The overall disc dimensions include an outer radius of about 6cm and an inner radius of about 0.75cm, wherein the disk is mounted on the spindle of a rotary device. The thickness of the disc ranged from about 0.9mm to about 1.5mm. The working fluid volume for reaction was about  $10\mu\text{L}$ .

The components of the mixing array were as follows. An entry port 901 having



a depth in the platform surface of about 0.75mm and lateral dimensions of about 0.5cm was constructed on the platform, and designed to accommodate a volume of about 10 $\mu$ L, ranging from 2 $\mu$ L to 20 $\mu$ L. This entry port was fluidly connected with a metering capillary 902 having cross-sectional diameter of about 0.5mm and having a depth of about 0.75mm; the length of this metering capillary was sufficient to contain a total volume of about 10 $\mu$ L. The metering capillary 902 was fluidly connected to capillary junction 904.

The entry port was also constructed to be fluidly connected with an overflow capillary 903 having a cross-sectional diameter of about 0.5mm and proximal ends rounded with respect to entry port 901. The overflow capillary was fluidly connected with an overflow chamber 905 having a depth in the platform surface of about 0.75mm, greater than the depth of the overflow capillary 903. Each of the overflow and fluid chambers was also connected with air ports or air channels, such as 523, that have dimensions of about 0.25mm deep and permit venting of air displaced by fluid movement on the platform. Capillary junctions 524 that were about 0.75mm deep were present in the air channels to prevent fluid flow into the air channels.

Entry port 901 was positioned on the platform 1.3cm from the center of rotation. Metering capillary 902 extends 4.2cm from entry port 901. Overflow capillary 902 extends from entry port 901 from about 1cm to about 20cm. The extent of the length of overflow capillary 903 was 20% longer than metering capillary 902. The position of overflow chamber 905 was from about 1cm to about 20cm from the center of rotation, and the position of capillary junction 904 was 5cm from the center of rotation.

Capillary junction 904 was fluidly connected with capillary channel 906, which in turn was fluidly connected with incubation chamber 910. Capillary channel 906 has a cross-sectional diameter of about 0.5mm and extends about 1cm. Incubation chamber 910 has a depth in the platform surface of about 0.75mm, that was greater than the depth of capillary channel 906. Capillary channel 906 was also fluidly connected with channel 909 through capillary junction 907. Capillary junction 907 was constructed to prevent fluid flow of the sample backwards through the junction and into the wash buffer. Channel 909 has a cross-sectional diameter of about 0.5mm and extends about 5cm. Capillary junction 907 has a depth in the platform surface of about 0.75mm, greater than

the depth of the channel 909 or capillary channel 906. Incubation chamber 910 also contains a specific binding species, most preferably an antibody, specific for a component of the sample. This species was advantageously contained within incubation chamber 910 as a coating on the surface of the chamber, or attached to beads or other carrier within the chamber, or to a functionalized inner surface of the chamber.

Capillary junction 907 was further fluidly connected with wash buffer reservoir 516, having a depth in the platform surface of about 0.75mm and positioned at a distance 3.7cm from the axis of rotation.

Capillary junction 907 was further fluidly connected with reagent capillary 920, which was further fluidly connected with capillary junction 914, which was further fluidly connected with channel 926, and which was fluidly connected with reagent reservoir 917. Reagent capillary 920 has a cross-sectional diameter of about 0.25mm and extended about 1cm. Capillary junction 914 has a depth in the platform surface of about 0.25mm and positioned at a distance of about 2.7cm from the axis of rotation.

Reagent capillary 926 has a cross-sectional diameter of about 0.25mm and extends from about 0.2cm to about 20cm. Reagent reservoir 917 has a depth in the platform surface of about 0.75mm and positioned at a distance of about 2.3cm from the axis of rotation.

Incubation chamber 910 was fluidly connected at a point most distal to the axis of rotation to U-shaped capillary 921. U-shaped capillary 921 has a cross-sectional diameter of about 0.5mm and extended about 1cm. This capillary extends in a U-shape to a point that was at least as proximal to the axis of rotation than the most axis-proximal extent of incubation chamber 910. This positioning of the U-shaped channel relative to incubation chamber 910 ensures that additional fluids flowing into incubation chamber 910 and displacing fluid therefrom will displace said fluid homogeneously, *i.e.*, the first fluid in the chamber will be pushed out of the chamber whilst being replaced by the second fluid.

This U-shaped capillary was also fluidly connected with waste reservoir 915. Waste reservoir 915 has a depth in the platform surface of about 0.75mm and positioned at a distance of about 4.5-5.7cm from the axis of rotation.

In certain embodiments of the invention, sacrificial valves 922 can be positioned at the junction of capillary junction 904 and capillary channel 906, at the junction of

capillary junction 907 and wash buffer capillary 908, or at the junction of reagent reservoir 918 and capillary junction 919.

As illustrated in Figures 27A through 27L, in the use of this platform reagent reservoir 916 and wash reservoir 915 were pre-loaded on the disk, and most preferably the disk contains sacrificial valves 922 at the junction of capillary junction 907 and wash buffer capillary 908, and at the junction of reagent reservoir 918 and capillary junction 919. An imprecise volume (ranging from 1-150 $\mu$ L of fluid) of a fluid was applied to the entry port 901 (Figure 27A). Fluid wicks into metering capillary 902 and stops at the capillary junction between metering capillary 902 and capillary junction 904 (Figures 27B and 27C). After sample loading by a user and filling of metering capillary 902 and overflow capillary 903 at no rotational speed, the platform was spun at a first rotational speed  $f_1$  of 45rpm.

Due to the greater distance of the end of overflow capillary 903 from the center of rotation than the end of metering capillary 902, fluid flows through overflow capillary 903 into overflow chamber 905 (Figure 27D). The platform was spun until all excess fluid was evacuated from entry port 901 and into overflow chamber 905, except the fluid contained in metering capillary 902 (Figure 27E).

At a second rotational speed  $f_2$  of 65rpm, the capillary junction 904 at the distal end of the metering capillary 902 was overcome, and sample from metering capillary 902 fills incubation chamber 910 (Figures 27F and 27G). A portion of the sample wicks into U-shaped capillary 914 to the level of the sample in incubation chamber 910 (Figure 27G). The sample was incubated for a time sufficient for maximum saturation binding of the component in the sample that specifically bind to the specific binding species.

At a third rotational speed  $f_3$  of 450rpm, the capillary junction 908 was overcome, and wash buffer from reservoir 916 flows through capillary 909, capillary 906, and into incubation chamber 910. Wash buffer fluid flow forces the sample through U-shaped capillary 914 and into waste reservoir 915 (Figures 27 H through 27J). Preferably, sacrificial valves 922 were released to permit wash buffer fluid flow.

At a fourth rotational speed  $f_4$  of 500rpm, the capillary junction 919 was overcome, and reagent buffer from reservoir 917 flows through capillary 918, capillary 920, capillary junction 908, capillary 906, and into incubation chamber 910. Reagent

buffer fluid flow forces the wash buffer through U-shaped capillary 914 and into waste reservoir 515 (Figures 27K through 27L). Preferably, sacrificial valves 922 were released to permit reagent buffer fluid flow.

5 The reagent buffer contained a chromogen or other developing agent for detection of specific binding in incubation chamber 910. The extent of specific immunoassay being was determined relative to the amount of chromogen produced in the assay.

## EXAMPLE 9

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### Immunoassays

Use of nitrocellulose for immobilizing antigens for use with the immunoassay arrays described in Example 8 were demonstrated as follows.

### Proof-of Principle

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Visual blue signals have been developed resulting from formation of a three-membered sandwich immobilized on a nitrocellulose (NC) surface. This sandwich is composed of (a) a capture anti-TSH monoclonal antibody (MAB) adsorbed onto porous NC; (b) TSH antigen, and c) a complimentary anti-TSH MAB coated onto colloidal blue latex particles. The intensity of blue color immobilized at the capture site increases in a regular way with antigen (TSH) level, thus providing a method of the quantitative analysis of unknown specimens.

20

Capture MAB was immobilized on 8- $\mu$ m NC by application of 2  $\mu$ L spots at a concentration of 10 mg/mL. These spots spread to circles about 1/4" in diameter. After brief incubation, the field surface of the NC was blocked in a bath of 1% BSA in PBS, followed by extensive washing with 0.1 % BSA-PBS. The membrane was allowed to air dry prior to use. The spotted region was then cut into small squares and introduced into glass test tubes followed by 200  $\mu$ L aliquots of serially diluted TSH standards in 50/50 horse serum/PBS-BSA buffer. After a brief incubation to wet the NC, 10  $\mu$ L of a 0.309 micron diameter blue latex suspension coated with a complimentary MAB was added and mixed. After 5 min of gentle shaking, the disk was removed, washed under a PBS-BSA stream, and allowed to dry. By visual inspection, the color intensity was

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seen to vary in the expected way with TSH concentration.

Additional antibody labeled groups, such a colloidal gold, fluorescent or colored latex beads, can be used to detect antibody binding and accumulation.

5      **Experimental: Competitive Immunoassay**

A competitive assay for the monovalent thyroid hormone Thyroxine has been developed analogous to and complimenting the sandwich assay described above for TSH. Antigen was immobilized on 5 $\mu$ m NC by application of 1.5  $\mu$ L volumes of BSA-T4 (12.5 mg/mL) followed by blocking with 1 % BSA in PBS and final washing with  
10      0.1 % BSA in PBS. Anti-thyroxine monoclonal antibody (MAB) was immobilized on Seradyne 0.309  $\mu$ m blue latex beads by passive adsorption.

Antibody-coated beads were briefly incubated in test tubes with T4 in buffer over a wide hormone concentration range. Square pieces of nitrocellulose containing adsorbed capture antigen were then added to the test tubes and incubated for 10 minutes,  
15      during which time color developed on the NC. The squares were then washed with a small volume of buffer and color intensity visually noted as a function of T4 level.

A roughly monotonic increase in blue color intensity with decreasing T4 concentration was found, as expected for a competitive assay. Change in color intensity occurred over a low T4 concentration range, implying that a serum specimen can be  
20      diluted by about ten-fold for analysis; *i.e.*, about 30 $\mu$ L of serum per test can be used, diluted to 300  $\mu$ L to produce detectable signal variation over the range of clinical diagnostic interest. A total assay time of about 20-?? minutes was determined without optimization, and low background and high signal intensity were obtained (without optimization). These results indicate that these procedures make it possible to detect  
25      immunoassay results at antigen and antibody concentrations useful for the detection of small amounts of antigen in complex mixtures, such as biological mixtures.

## EXAMPLE 10

### **Preparation of Resistive Heater Elements**

30      Resistive heater elements were prepared on the platforms of the invention as follows. Portions of the surface of a microsystems platform on which a resistive heater

element are desired are screen printed with a resistive ink electrically coupled with a conductive ink to conduct electricity across the resistive ink in the presence of a direct current (DC) voltage drop across the extent of the resistive ink. Both the resistive ink and the conductive ink are screened printed using methods well known in the art (Gilleo, *ibid.*)

Briefly, a conductive ink pattern, such as those illustrated in Figure 30, was screened onto a heat stabilized polyester sheet substrate (ICI ST505), and cured at 110-120°C for 10 minutes. A resistive ink was then screened over the cured conductive ink pattern and the composite cured for an additional 10 min at 110-120°C. Typically, the ink is laid down in a layer that is about 10 microns thick, over an area at least 0.5mm x 0.5mm (0.25mm<sup>2</sup>). However, film patterns of greater thickness have been produced by printing, curing and reprinting the same polyester sheet in the same pattern up to three times. It was found that these thicker films had reduced resistance and increased heating ability at the same applied voltage.

In some instances, polymer thick films are covered with a dielectric layer that insulates the electrical circuit; this is particularly advantageous in embodiments of the resistive heaters that are used to heat liquids on the platforms of the invention. This dielectric layer is also deposited by screen printing and then cured using ultraviolet radiation for several seconds at 1000mJ/cm<sup>2</sup>.

Figures 30 and 31 show an example of a circuit that was screen printed onto heat stabilized polyester. The circuit materials consisted of Dupont 5028 conductive ink and Dupont 7082 resistive ink; the resistive ink comprised the solid back patterned areas and the conductive inks comprised the light, thin lined patterns on the disc. Electrical connection to a DC power source are labeled as 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, and 22. Resistances of the different circuit elements ranged from approximately 10 to approximately 200 Ohms, depending on circuit geometry. For example, a resistance of  $12.1 \pm 1.2$  Ohm ( $N = 10$ ) was measured between electrical contacts 11 and 12. In another example, a resistance of  $1808 \pm 257$  Ohm ( $N = 10$ ) was measured between electrical contacts 11 and 22.

In another experiment, Dupont 7082 resistive ink (400 Ohm per square per mil) was blended with Dupont 7102 resistive ink (20 Ohm per square per mil). In a 50 : 50

mixture (by wet weight) and then screen printed into the patterns shown in Figures 30 and 31, a resistance of  $7.3 \pm 0.6 \text{ Ohm}$  ( $N=7$ ) was measured between electrical contacts 11 and 12. In another example, a resistance of  $272.2 \pm 22.7 \text{ Ohm}$  ( $N=7$ ) was measured between electrical contacts 11 and 22.

5           When resistive heater elements were further screen printed, cured, reprinted and re-cured over the same region with Dupont 7082 resistive ink, the resistive circuits were thickened and the resistance was decreased. In this experiment, a second and third printing reduced the resistance between electrical contacts 11 and 12 to  $679 \pm 86.5 \text{ Ohm}$  ( $N=8$ ). Thus, the ability to tailor resistance through appropriate choice of ink  
10           formulation and reprinting of resistive circuits enables control of the electrical properties of the final screen printed circuit.

#### EXAMPLE 11

##### Use of Resistive Polymer Thick Films as Heater Elements

15           The use of resistive polymer thick films as heater elements depends on the capacity of resistive polymer thick films to produce heat when an electrical potential is applied across a resistive polymer thick film element produced as described in Example 10. The capacity of a resistive polymer thick film element to be used as a heater is shown in Figure 32. Circuits in the pattern shown in Figures 30 and 31 were screen-  
20           printed using Dupont 5028 silver paste as the conductive electrode combined with a 50 : 50 mixture of Dupont 7082 resistive ink blended with Dupont 7102 resistive ink to form the resistive heating element. A direct current (DC) voltage was applied across the electrical contacts 31 and 33 in Figure 31 and the temperature at the surface of the heater measure using a thermistor probe. These experiments were performed "dry", *that is*, on  
25           a resistive heater element without application of a fluid on the platform, and stationary, *that is*, without rotation of the disc. Figure 32 is a graph of the temperature produced as a function of time and applied (DC) voltage (voltage applied was 2V, 3V, 4V, 5V, and 6V, read bottom to top from the voltage that produced the lowest steady-state voltage through the highest steady-state voltage). This graph shows that the maximum  
30           temperature produced increased with both time and applied voltage, and that the maximum voltage reached a steady state within between 100-150 seconds, and that the

maximum voltage achieved was about 85-90°C. These data have been converted into the graph shown in Figure 33, which show a plot of the steady-state temperature obtained as a function of applied (DC) voltage. These results show that the steady-state temperature achieved increases in a parabolic fashion with increased applied voltage.

5           The dependence of maximum steady-state temperature on voltage was also determined using positive temperature coefficient (PTC) inks, specifically Dupont 7285 ink, in experiments performed as described above. The voltage dependence on temperature is linear over the voltage range tested, and the applied (DC) voltages were about ten-fold higher than the results obtained using blended resistive inks.

10           These results are contrasted in Figure 33 with the results obtained with PTC inks, which approach a steady state temperature with increasing voltage, as expected for a positive temperature controlled ink. The results obtained with PTC inks were obtained using a resistive heating element screen-printed in a patterns as shown in Figure 31 using Dupont 7285 PTC ink.

15           The temperature of the substrate at increasing distance from the resistive heater elements of the invention was determined. These results are shown in Figure 34, where the temperature of the disk drops to ambient within 1-2mm of the resistive heater. These results indicate that resistive heaters can be arrayed in close proximity without the activation of one of the heaters affecting either the adjacent heater or the platform component (such as a sacrificial valve) controlled by the adjacent heater.

20           These results demonstrate the capacity of resistive elements prepared using polymer thick films according to the teachings of this invention to be used as resistive heater elements on synthetic substrates appropriate for the production of microsystems platforms of this invention.

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## EXAMPLE 12

### Use of Resistive Heaters with Heat-Activated Valves

30           Resistive heater elements prepared using resistive polymer thick films as described in Examples 10 and 11 are advantageously used to activate heat-activated valves. Heat-activated valves include valves prepared by depositing a "wax" in a



channel or capillary of the fluidics structures disclosed herein.

In preparing heat-activated wax valves, a small amount of molten wax is taken up in a preheated plastic pipette sip, and when the tip is applied to a capillary channel, the channel takes up some of the molten wax by capillary action. The wax valve forms when the wax cools and solidifies in the channel or capillary. Examples of particular hydrocarbons useful in preparing wax valves include the monodisperse alkanes eicosane ( $T_m = 36.8^\circ\text{C}$ ), tetracosane ( $T_m = 54.0^\circ\text{C}$ ), and octocosane ( $T_m = 64.5^\circ\text{C}$ ), as well as polydisperse waxes such as paraffin ( $T_m = 54.4^\circ\text{C}$ ). Experiments using these different waxes showed that paraffin is preferred over the monodisperse hydrocarbons because it is easier to work. Use of temperature-controlled dispensing tips could avoid this distinction and permit monodisperse hydrocarbons to be advantageously used as wax valves.

After production of wax valves as described above, a resistive heater layer is prepared on a polyester substrate and bonded to the platform substrate with tape. Heater positioning is important to ensure that the wax plug is fully melted, is capable of even flow and will not re-crystallize and clog the channel or capillary downstream of the location of the resistive heater.

In an alternative embodiment, the channels and capillaries of the invention comprising wax valves also comprise wax recrystallization chambers, illustrated in Figures 35 and 37, positioned "downstream" (*that is*, more proximal to the edge of the platform) from the resistive heater. When the disc is assembled and spinning, the melted wax flows into the recrystallization chamber and solidifies, typically on the walls of the recrystallization chamber. It has been found that optimal functioning of such wax valves requires that the resistive heating element be positioned over the entire extent of the wax valve and over at least the interior 25% of the wax recrystallization chamber.

Figures 36A through 36C provide an illustration of how a platform comprising the wax valves, fluidics structures and resistive polymer thick films is prepared. As shown in Figure 36A, heater circuits are screen printed on polyester films bonded to a polycarbonate disc with double-sided tape. Fluidics structures are machined onto an acrylic disc as described herein and shown in Figure 36B. Wax valves positioned manually at appropriate regions of the fluidics channels and capillaries of the platforms

of the invention. These two components are then bonded with double-sided tape to form the assembled disc (shown in Figure 36C)..

Figure 37 is a more detailed and expanded view of one of the fluid handling arrays of the invention. The screen-printed lead, prepared according to Example 10, is placed on the platform so that the wax valve is completely covered with the screen printed heater element, which also extends over a portion of the wax recrystallization chamber.

Figure 38 illustrates how the heat-activated wax valves can be used to sequentially open the wax valves and permit fluid flow from a fluid chamber into a fluid receptacle. Aqueous dye was loaded into each of the fluid chambers shown in Figure 38. The disc was rotated at about 700 rpm, a speed above the capillary burst rpm. The Figure illustrates that sequential application of 10V DC across these resistive heaters is sufficient to completely melt the wax valves without reclosure of the wax valves. Also, the heating and melting of any one of these wax valves was insufficient to cause melting of any of the adjacent wax valves, which are separated on the platform by about 1-2mm.

### EXAMPLE 13

#### Use of Resistive Heaters with Heat-Activated Valves: First Alternative

In an alternative embodiment, heat-recoverable polymers were used as heat activated valves. In this experiment, heat recoverable tubing FP301H (obtained from 3M, Minneapolis, MN) was cut into a sheet and placed in a capillary channel on a microsystems platform of the invention. This capillary channel divided two fluid chambers, the interior-most one of which contained 25-50 $\mu$ L of fluid. The heat-recoverable polymer functioned as a fluid-tight valve, and no leakage of fluid was observed into the second fluid chamber. The disc was then heated to approximately 100°C and the heat recoverable tubing was observed to shrink by approximately 50%, and liquid was observed to be transported through the channel to the second, exterior fluid chamber.

These results demonstrate that heat-recoverable polymers can be advantageously used as a sacrificial valve. A particular advantage of this type of valve when compared with a wax valve is that the polymer sheet is macroscopic object that is retained by the

channel, most likely without clogging either the channel or any microfluidics structure downstream from the sacrificial valve site.

#### EXAMPLE 14

##### 5      Use of Resistive Heaters with Heat-Activated Valves: Second Alternative

The use of the resistive heater itself as a sacrificial valve was demonstrated. It was observed that certain configurations of the resistive heaters of the invention were capable of melting the platform substrate when sufficient voltages were placed across the resistive heater. In this experiment, 15V (DC) was applied across the heaters shown  
10      in Figure 31. After less than 1 second at this voltage, a hole developed within the resistive element itself, typically at its center, having an area of about 1mm<sup>2</sup>. These results suggested that the heater could be used as a sacrificial valve. In this embodiment, a platform comprising a chamber is prepared connected to a channel that terminates at a radius R from the center of rotation. This terminal part of the channel is then bonded  
15      to a heater circuit positioned just below the channel termination point. A second fluidics disk is prepared having a channel positioned at precisely the termination point of the first channel, and is then bonded to this heater circuit so that the beginning point of this second channel is positioned just below the resistive element. After bonding the platform layers together, the resistive element will be positioned between the two  
20      channels in different layers of the platform, so that heating through the element will conjoin the two channels and permit fluid flow from one chamber to the other at a different layer of the platform.

#### EXAMPLE 14

##### 25      Use of Resistive Heaters to Heat a Fluid

The resistive heaters of the invention are also used to heat a fluid in an incubation chamber or other microfluidics component of a platform of the invention. A DC voltage of 40V was applied between contacts 31 and 33 in a resistive heater produced as shown in Figure 31, in which a fluid chamber containing about 65 $\mu$ L of water was placed in  
30      thermal contact. A thermistor was also placed in the fluid chamber to monitor the temperature of the water, and the platform was rotated at about 500rpm. These results

are shown in Figure 39. A steady-state temperature was obtained after about 200 seconds. After about 500 seconds, the heater was turned off while rotation of the platform continued. The results shown in Figure 39 demonstrate that a resistive heater element of the invention was able to heat this water sample to about 50°C and maintain this temperature for almost 10 minutes, and that convective cooling of the spinning platform reduced the temperature to ambient within 1-2 minutes of the resistive heater shut-off.

This experiment also demonstrated that temperature can be rapidly cycled in a rotating platform by sequential activation of a resistive heater element.

#### EXAMPLE 14

##### Use of Resistive Heaters as Thermosensors

Because screen-printed PTC inks display a larger change in resistance with temperature than other inks, these inks can be used to measure temperature on a screen-printed circuit.

In this embodiment, the temperature-sensing device is screen-printed identically as a resistive heater element; however, rather than applying a voltage across the element to produce heat, the resistance of the element is sampled to determine whether resistance has increase as a result of heating. In one application, the resistive heater element itself could be used both as a heater and a temperature sensing device. In this use, the heater circuit is coupled to an external circuit that is switched between constant voltage and constant (small) current modes. In the constant voltage mode the element would heat up, and in the constant current mode the voltage drop would be measured and the temperature determined thereby.

In another application, a PTC ink is screen-printed over a portion to a resistive heater element. The resistive heater and PTC ink/thermosensor elements are joined to external circuitry using different sets of conductive leads; the external circuit delivers voltage to the resistive heater element and a small constant current to the thermosensor element. By measuring the voltage drop in the PTC ink element, the temperature of the resistive element is inferred. Coordination of these two circuits could be provided so

that the thermosensor is used to regulate the temperature of the resistive heating element.

5 It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention.

**What is claimed is:**

1. A microsystem platform comprising
  - a) a rotatable platform, comprising a substrate having a first flat, planar surface and a second flat, planar surface opposite thereto, each surface comprising a center about which the platform is rotated, wherein the first surface comprises in combination
  - b) an entry port comprising a depression in the first surface having a volumetric capacity of about 1 to about 150 $\mu$ L, that is fluidly connected with
  - c) a first metering capillary array comprising a multiplicity of metering capillaries, and an overflow capillary, each being fluidly connected with the entry port, wherein each capillary defines a cross-sectional area of about 0.02mm to about 1mm in diameter, and wherein each capillary extends radially from the center of the platform and defines a first end proximally arrayed towards the center of the platform and a second end distally arrayed from the center of the platform, wherein the proximal end of each capillary defines a curved opening; wherein the first metering capillary array defines a volume of the fluid and wherein the first metering capillary array is fluidly connected with
  - d) a first fluid chamber having a depth in the surface of the platform equal to or greater than the metering capillary and positioned radially more distant from the center of the platform than the entry port, and wherein the overflow capillary is fluidly connected with
  - e) an overflow chamber having a depth in the surface of the platform equal to or greater than the overflow capillary and positioned radially more distant from the center of the platform than the holding channel and the entry port,

wherein a capillary junction is formed at the junction of each of the metering capillaries comprising the metering capillary array and the first fluid chamber and at the junction of the overflow capillary and the overflow chamber, whereby fluid placed onto the disk at the entry port flows by capillary action to the junction of each of the metering capillaries comprising the metering capillary array and the first fluid chamber, and

excess fluid flows by capillary action to the junction of the overflow capillary and the overflow chamber; and wherein rotation of the platform at a first rotation speed motivates fluid displacement in the overflow capillary into the overflow chamber but not fluid displacement in any of the metering capillaries comprising the metering capillary array, whereby rotation of the platform at the first rotational speed drains the fluid from the entry port into the overflow chamber; and

wherein rotation of the platform at a second rotation speed that is greater than the first rotational speed motivates fluid displacement of the volume of the fluid in the metering capillary array into the first fluid chamber; and wherein each of the first fluid chamber and overflow chamber also comprise air displacement channels whereby air displaced by fluid movement is vented to the surface of the platform.

2. The microsystem platform of Claim 1 further comprising
6. a capillary having a first end fluidly connected to the first fluid chamber and having a second end fluidly connected to a holding chamber, having a depth in the surface of the platform equal to or greater than the capillary and positioned radially more distant from the center of the platform than the first fluid chamber,

wherein a capillary junction is formed at the junction of the capillary and the holding chamber, whereby fluid in the first fluid chamber flows through the capillary to the junction of the capillary and the holding chamber; and wherein rotation of the platform at a third rotational speed that is greater than the second rotational speed motivates fluid displacement of the volume of the fluid in the first fluid chamber into the holding chamber and wherein the holding chamber also comprises air displacement channels whereby air displaced by fluid movement is vented to the surface of the platform.

3. The microsystem platform of Claim 2 further comprising
1. a capillary having a first end fluidly connected to the holding chamber and having a second end fluidly connected to a read chamber, having a depth in the surface of the platform equal to or greater than the capillary and positioned radially more distant from the center of the platform than the holding chamber,

wherein a capillary junction is formed at the junction of the capillary and the read chamber, whereby fluid in the holding chamber flows through the capillary to the junction of the capillary and the read chamber; and wherein rotation of the platform at a fourth rotational speed that is greater than the third rotational speed motivates fluid displacement of the volume of the fluid in the holding chamber into the read chamber and wherein each of the holding chamber and the read chamber also comprises air displacement channels whereby air displaced by fluid movement is vented to the surface of the platform..

4. The microsystem platform of Claim 3, further comprising
- b) a sacrificial valve in the capillary extending from the holding chamber to the read chamber, wherein release of the sacrificial valve permits fluid flow from the holding chamber to the read chamber at a non-zero rotational speed.

5. The microsystem platform of Claim 4 wherein the sacrificial valve is a solid, semi-solid or viscous liquid hydrocarbon, or a plastic.

6. The microsystem platform of Claim 5 further comprising a heating element in the platform in thermal contact with the sacrificial valve, wherein heating the heating element releases the sacrificial valve.

7. The microsystem platform of Claim 3, wherein the holding chamber comprises a first component of a biological detection assay and the read chamber comprises a second component of a biological detection assay, wherein a sample is assayed for the presence of an analyte.

8. The microsystem platform of Claim 7, wherein the holding chamber comprises carboxypeptidase and a peptide comprising a D-amino acid at its carboxyl terminus, and the read chamber comprises D-amino acid oxidase, flavine adenine dinucleotide, horseradish peroxidase and a chromogen, and the biological detection assay detects the presence of  $\beta$ -lactam antibiotics in a fluid sample.



9. The microsystem platform of Claim 8 wherein the fluid sample is milk.

10. A method for moving a fluid in a microsystem platform according to Claim 1, the method comprising the steps of

- 5
- a) applying an amount of a fluid sample comprising a volume of about 1 to about 150 $\mu$ L to the entry port of the rotatable microsystem platform;
  - b) rotating the platform at a first rotation speed for a time sufficient to displace the fluid in the entry port and the overflow capillary into the overflow chamber;
  - 10 c) rotating the platform at a second rotation speed that is greater than the first rotational speed to displace a volume of the fluid in the metering capillary into the first fluid chamber.

11. A method for moving a fluid in a microsystem platform according to Claim 2, the method comprising the steps of

- a) applying an amount of a fluid sample comprising a volume of about 1 to about 150 $\mu$ L to the entry port of the rotatable microsystem platform;
- b) rotating the platform at a first rotation speed for a time sufficient to displace the fluid in the entry port and the overflow capillary into the overflow chamber;
- c) rotating the platform at a second rotation speed that is greater than the first rotational speed to displace a volume of the fluid in the metering capillary into the first fluid chamber; and
- c) rotating the platform at a third rotational speed that is greater than the second rotational speed to displace a volume of the fluid in the first fluid chamber into the holding chamber.

12. A method for moving a fluid in a microsystem platform according to Claim 3, the method comprising the steps of

- a) applying an amount of a fluid sample comprising a volume of about 1 to about 150 $\mu$ L to the entry port of the rotatable microsystem platform;
- b) rotating the platform at a first rotation speed for a time sufficient to

displace the fluid in the entry port and the overflow capillary into the overflow chamber;

- c) rotating the platform at a second rotation speed that is greater than the first rotational speed to displace a volume of the fluid in the metering capillary into the first fluid chamber;
- d) rotating the platform at a third rotational speed that is greater than the second rotational speed to displace a volume of the fluid in the first fluid chamber into the holding chamber; and
- e) rotating the platform at a fourth rotational speed that is greater than the second rotational speed to displace a volume of the fluid in the holding chamber into the read chamber.

13. A method for detecting an amount of a  $\beta$ -lactam antibiotic in a fluid sample using a microsystem platform according to Claim 8, the method comprising the steps of

- a) applying an amount of a fluid sample comprising a volume of about 1 to about 150 $\mu$ L to the entry port of the rotatable microsystem platform;
- b) rotating the platform at a first rotation speed for a time sufficient to displace the fluid in the entry port and the overflow capillary into the overflow chamber;
- c) rotating the platform at a second rotation speed that is greater than the first rotational speed to displace a volume of the fluid in the metering capillary into the first fluid chamber;
- d) rotating the platform at a third rotational speed that is greater than the second rotational speed to displace a volume of the fluid in the first fluid chamber into the holding chamber;
- e) incubating the fluid in the holding chamber for a time and at a temperature sufficient to inhibit carboxypeptidase activity;
- f) rotating the platform at a fourth rotational speed that is greater than the second rotational speed to displace a volume of the fluid in the holding chamber into the read chamber;
- g) incubating the fluid in the read chamber for a time and at a temperature

- sufficient to develop the chromogen;
- h) detecting the amount of developed chromogen in the read chamber and comparing said amount with the amount produced by a sample that does not contain a  $\beta$ -lactam antibiotic.
14. A microsystem platform comprising
- a) a rotatable platform, comprising a substrate having a first flat, planar surface and a second flat, planar surface opposite thereto, each surface comprising a center about which the platform is rotated, wherein the first surface comprises in combination
- b) an entry port comprising a depression in the first surface having a volumetric capacity of about 1 to about 150  $\mu$ L, that is fluidly connected with
- c) a capillary array comprising a multiplicity of capillaries, each capillary being fluidly connected with the entry port, wherein each capillary defines a cross-sectional area of about 0.02mm to about 1mm in diameter, and wherein each capillary extends radially from the center of the platform and defines a first end proximally arrayed towards the center of the platform and a second end distally arrayed from the center of the platform, wherein the proximal end of each capillary defines a curved opening; wherein the capillary array defines a volume of the fluid and wherein the capillary array is fluidly connected with
- d) a first fluid chamber having a depth in the surface of the platform equal to or greater than the metering capillary and positioned radially more distant from the center of the platform than the entry port, and wherein the first fluid chamber is also fluidly connected with an overflow capillary that is fluidly connected with
- e) an overflow chamber having a depth in the surface of the platform equal to or greater than the overflow capillary and positioned radially more distant from the center of the platform than the holding channel and the entry port, wherein the overflow capillary is fluidly connected with the first fluid chamber at a position on the platform that is closer to the axis

of rotation than the position on the platform where the capillaries of the capillary array are fluidly connected with the first fluid chamber; wherein a capillary junction is formed at the junction of each of the capillaries comprising the capillary array and the first fluid chamber and at the junction of the overflow capillary and the overflow chamber, whereby fluid placed onto the disk at the entry port flows by capillary action to the junction of each of the capillaries comprising the capillary array and the first fluid chamber, and wherein rotation of the platform at a first rotation speed motivates fluid displacement of the volume of the fluid in entry port through the capillary array into the first fluid chamber until the level of the fluid in the first fluid chamber reaches the position of the fluid connection between the first fluid chamber and the overflow capillary, wherein excess fluid flows through the overflow capillary to the capillary junction with the overflow chamber at the first rotational speed, and drains excess fluid from the first fluid chamber through the overflow channel and into the overflow chamber, until the level of fluid in the first fluid chamber is decreased to a level farther from the center of rotation in the first fluid chamber than the position where the overflow capillary is fluidly connected with the chamber, thereby leaving a defined volume of fluid in the first fluid chamber; and wherein each of the first fluid chamber and overflow chamber also comprise air displacement channels whereby air displaced by fluid movement is vented to the surface of the platform.

15. The microsystem platform of Claim 14 further comprising
  - f) a second capillary having a first end fluidly connected to the first fluid chamber and having a second end fluidly connected to a holding chamber, having a depth in the surface of the platform equal to or greater than the capillary and positioned radially more distant from the center of the platform than the first fluid chamber,

wherein a capillary junction is formed at the junction of the capillary and the holding chamber, whereby fluid in the first fluid chamber flows through the capillary to the junction of the capillary and the holding chamber during rotation at the first rotational speed; and wherein rotation of the platform at a second rotational speed that is greater than the first rotational speed motivates fluid displacement of the volume of the fluid in

the first fluid chamber into the holding chamber and wherein the holding chamber also comprises air displacement channels whereby air displaced by fluid movement is vented to the surface of the platform.

16. The microsystem platform of Claim 15 further comprising
- g) a capillary having a first end fluidly connected to the holding chamber and having a second end fluidly connected to a read chamber, having a depth in the surface of the platform equal to or greater than the capillary and positioned radially more distant from the center of the platform than the holding chamber,

wherein a capillary junction is formed at the junction of the capillary and the read chamber, whereby fluid in the holding chamber flows through the capillary to the junction of the capillary and the read chamber; and wherein rotation of the platform at a third rotational speed that is greater than the second rotational speed motivates fluid displacement of the volume of the fluid in the holding chamber into the read chamber and wherein each of the holding chamber and the read chamber also comprises air displacement channels whereby air displaced by fluid movement is vented to the surface of the platform.

17. The microsystem platform of Claim 16, further comprising
- h) a sacrificial valve in the capillary extending from the holding chamber to the read chamber, wherein release of the sacrificial valve permits fluid flow from the holding chamber to the read chamber at a non-zero rotational speed.

18. The microsystem platform of Claim 17 wherein the sacrificial valve is a solid, semi-solid or viscous liquid hydrocarbon, or a plastic.

19. The microsystem platform of Claim 18 further comprising a heating element in the platform in thermal contact with the sacrificial valve, wherein heating the heating element releases the sacrificial valve.

20. The microsystem platform of Claim 16, wherein the holding chamber comprises a first component of a biological detection assay and the read chamber comprises a second component of a biological detection assay, wherein a sample is assayed for the presence of an analyte.

21. The microsystem platform of Claim 20, wherein the holding chamber comprises carboxypeptidase and a peptide comprising a D-amino acid at its carboxyl terminus, and the read chamber comprises D-amino acid oxidase, flavine adenine dinucleotide, horseradish peroxidase and a chromogen, and the biological detection assay detects the presence of  $\beta$ -lactam antibiotics in a fluid sample.

22. The microsystem platform of Claim 21 wherein the fluid sample is milk.

23. A method for moving a fluid in a microsystem platform according to Claim 14, the method comprising the steps of

- a) applying an amount of a fluid sample comprising a volume of about 1 to about 150 $\mu$ L to the entry port of the rotatable microsystem platform;
- b) rotating the platform at a first rotation speed for a time sufficient to displace the fluid in the entry port and the capillary array into the first fluid chamber and the overflow capillary; and
- c) rotating the platform at a said first rotational speed for a time sufficient to displace an excess volume of the fluid in the first fluid chamber into the overflow chamber.

24. A method for moving a fluid in a microsystem platform according to Claim 15, the method comprising the steps of

- a) applying an amount of a fluid sample comprising a volume of about 1 to about 150 $\mu$ L to the entry port of the rotatable microsystem platform;
- b) rotating the platform at a first rotation speed for a time sufficient to displace the fluid in the entry port and the capillary array into the first fluid chamber, the second capillary and the overflow capillary;
- c) rotating the platform at a said first rotational speed for a time sufficient

to displace an excess volume of the fluid in the first fluid chamber into the overflow chamber; and

- d) rotating the platform at a third rotational speed that is greater than the second rotational speed to displace a volume of the fluid in the first fluid chamber into the holding chamber.

25. A method for moving a fluid in a microsystem platform according to Claim 16, the method comprising the steps of

- a) applying an amount of a fluid sample comprising a volume of about 1 to about 150 $\mu$ L to the entry port of the rotatable microsystem platform;
- b) rotating the platform at a first rotation speed for a time sufficient to displace the fluid in the entry port and the capillary array into the first fluid chamber, the second capillary and the overflow capillary;
- c) rotating the platform at a said first rotational speed for a time sufficient to displace an excess volume of the fluid in the first fluid chamber into the overflow chamber;
- d) rotating the platform at a second rotational speed that is greater than the first rotational speed to displace a volume of the fluid in the first fluid chamber into the holding chamber; and
- e) rotating the platform at a third rotational speed that is greater than the second rotational speed to displace a volume of the fluid in the holding chamber into the read chamber.

26. A method for detecting an amount of a  $\beta$ -lactam antibiotic in a fluid sample using a microsystem platform according to Claim 21, the method comprising the steps of

- a) applying an amount of a fluid sample comprising a volume of about 1 to about 150 $\mu$ L to the entry port of the rotatable microsystem platform;
- b) rotating the platform at a first rotation speed for a time sufficient to displace the fluid in the entry port and the capillary array into the first fluid chamber, the second capillary and the overflow capillary;
- c) rotating the platform at a said first rotational speed for a time sufficient

to displace an excess volume of the fluid in the first fluid chamber into the overflow chamber; and

- d) rotating the platform at a second rotational speed that is greater than the first rotational speed to displace a volume of the fluid in the first fluid chamber into the holding chamber;
- e) incubating the fluid in the holding chamber for a time and at a temperature sufficient to inhibit carboxypeptidase activity;
- f) rotating the platform at a third rotational speed that is greater than the second rotational speed to displace a volume of the fluid in the holding chamber into the read chamber;
- g) incubating the fluid in the read chamber for a time and at a temperature sufficient to develop the chromogen;
- h) detecting the amount of developed chromogen in the read chamber and comparing said amount with the amount produced by a sample that does not contain a  $\beta$ -lactam antibiotic.

27. A microsystem platform comprising

- a) a rotatable platform, comprising a substrate having a first flat, planar surface and a second flat, planar surface opposite thereto, each surface comprising a center about which the platform is rotated, wherein the first surface comprises in combination
- b) an entry port comprising a depression in the first surface having a volumetric capacity of about 1 to about 50  $\mu$ L, that is fluidly connected with
- c) an entry capillary that defines a cross-sectional area of about 0.1 to about 2 cm in diameter and having a volume of about 5 to 25  $\mu$ L, wherein the capillary extends radially from the center of the platform and defines a first end proximally arrayed towards the center of the platform and a second end distally arrayed from the center of the platform, wherein the proximal end of each capillary defines a curved opening; wherein the entry capillary is fluidly connected with
- d) a separation column having a cross-sectional diameter of about 0.1 to



about 2cm and having a volume of about 5 to 25  $\mu\text{L}$ , and having a depth in the surface of the platform equal to or greater than the entry capillary, and that defines a first, closed end proximally arrayed towards the center of the platform and a second closed end distally arrayed from the center of the platform, wherein the separation chamber is positioned substantially parallel to the entry capillary and wherein the entry capillary is fluidly connected with the separation chamber at a position on the platform that is substantially more proximal to the second end of the chamber than the first end of the chamber;

wherein a capillary junction is formed at the junction of the entry capillary and the separation chamber, whereby fluid placed onto the disk at the entry port flows by capillary action to the junction of the entry capillary and the separation chamber, and wherein rotation of the platform at a first rotation speed motivates fluid displacement in the entry capillary into the separation; and wherein the separation chamber also comprises air displacement channels whereby air displaced by fluid movement is vented to the surface of the platform.

28. The microsystem platform of Claim 27 further comprising
- c) an overflow chamber fluidly connected to the separation chamber by a channel, wherein the position of the channel on the platform is substantially more proximal to the first end of the chamber than the second end of the chamber;

and wherein the overflow chamber also comprises air displacement channels whereby air displaced by fluid movement is vented to the surface of the platform.

29. The microsystem platform of Claim 28 further comprising
- f) an capillary fluidly connected to the separation chamber at a first end and having a cross-sectional diameter of about 0.05mm to about 0.25mm and a second end fluidly connected with a capillary junction having a depth in the surface of the platform greater than the depth of the capillary, wherein the capillary junction is fluidly connected with
  - g) a decant capillary having a cross-sectional diameter of about 0.25mm to

about 1 mm, wherein the capillary extends radially from the center of the platform and defines a first end proximally arrayed towards the center of the platform and a second end distally arrayed from the center of the platform, wherein the second end of the decant capillary is fluidly connected with

- h) a decant chamber having a depth in the surface of the platform equal to or greater than the capillary and positioned radially more distant from the center of the platform than the capillary junction,

wherein rotation of the platform at a rotational speed greater than the first rotational speed motivates fluid flow through the capillary junction and into the decant chamber, and wherein the decant chamber also comprises air displacement channels whereby air displaced by fluid movement is vented to the surface of the platform.

30. A method for separating a fluid from a suspension of particulate in said fluid using a microsystem platform according to Claim 27, the method comprising the steps of

- a) applying an amount of a fluid sample comprising a particulate suspension having a volume of about 1 to about 50  $\mu$ L to the entry port of the rotatable microsystem platform and allowing the fluid to fill the entry capillary;
- b) rotating the platform at a first rotation speed for a time sufficient to displace the fluid in the entry port into the separation chamber; and
- c) rotating the platform at a second rotation speed that is greater than the first rotational speed for a time sufficient to cause the particulates in the suspension to be concentrated in a portion of the separation chamber that is closer to the second end of the chamber than the first end of the chamber.

31. A method for separating a fluid from a suspension of particulate in said fluid using a microsystem platform according to Claim 28, the method comprising the steps of

- a) applying an amount of a fluid sample comprising a particulate suspension

having a volume of about 1 to about 50 $\mu$ L to the entry port of the rotatable microsystem platform and allowing the fluid to fill the entry capillary;

- b) rotating the platform at a first rotation speed for a time sufficient to displace the fluid in the entry port into the separation chamber and for excess fluid to be displaced into the overflow chamber; and
- c) rotating the platform at a second rotation speed that is greater than the first rotational speed for a time sufficient to cause the particulates in the suspension to be concentrated in a portion of the separation chamber that is closer to the second end of the chamber than the first end of the chamber.

32. A method for separating a fluid from a suspension of particulate in said fluid using a microsystem platform according to Claim 29, the method comprising the steps of

- a) applying an amount of a fluid sample comprising a particulate suspension having a volume of about 1 to about 50 $\mu$ L to the entry port of the rotatable microsystem platform and allowing the fluid to fill the entry capillary;
- b) rotating the platform at a first rotation speed for a time sufficient to displace the fluid in the entry port into the separation chamber and for excess fluid to be displaced into the overflow chamber;
- c) rotating the platform at a second rotation speed that is greater than the first rotational speed for a time sufficient to cause the particulates in the suspension to be concentrated in a portion of the separation chamber that is closer to the second end of the chamber than the first end of the chamber; and
- d) rotating the platform at a third rotational speed that is greater than the second rotational speed to motivate displacement of fluid from the separation column through the capillary junction and the decant capillary and into the decant chamber, wherein the fluid is substantially free of particulates.

33. A method according to Claim 30 wherein the fluid is blood
34. A method according to Claim 31 wherein the fluid is blood
35. A method according to Claim 32 wherein the fluid is blood
36. A microsystem platform of Claim 29 wherein the particulate suspension has a viscosity that prevents fluid flow through the second capillary.
37. A microsystem platform comprising
  - a) a rotatable platform, comprising a substrate having a first flat, planar surface and a second flat, planar surface opposite thereto, each surface comprising a center about which the platform is rotated, wherein the first surface comprises in combination
  - b) an entry port comprising a depression in the first surface having a volumetric capacity of about 5 to about 50 $\mu$ L, that is fluidly connected with
  - c) an overflow capillary that is fluidly connected with
  - d) an overflow chamber having a depth in the surface of the platform equal to or greater than the overflow capillary and positioned radially more distant from the center of the platform than the entry port, and wherein the entry port is fluidly connected with
  - e) a first metering capillary array and a metering second capillary array, each comprising a multiplicity of capillaries, each capillary being fluidly connected with the entry port, wherein each capillary defines a cross-sectional area of about 0.02mm to about 1mm in diameter, and wherein each capillary extends radially from the center of the platform and defines a first end proximally arrayed towards the center of the platform and a second end distally arrayed from the center of the platform, wherein the proximal end of each capillary defines a curved opening; wherein the capillary array defines a volume of the fluidand wherein the first metering capillary array is fluidly connected with
  - f) a ballast chamber having a depth in the surface of the platform equal to

or greater than the metering capillary and positioned radially more distant from the center of the platform than the entry port but radially less distant than the capillary junction, wherein a capillary junction is formed at the junction of each of the capillaries comprising the second metering capillary array and the ballast chamber;

and wherein the second metering capillary array is fluidly connected with

- g) a capillary junction having a depth in the surface of the platform equal to or greater than the metering capillary and positioned radially more distant from the center of the platform than the entry port; and wherein the capillary junction is fluidly connected to
- h) a channel that is fluidly connected to
- i) a separation chamber that is positioned radially more distant from the center of the platform than the ballast chamber and having a first end proximally arrayed towards the center of the platform and a second end distally arrayed from the center of the platform;

whereby fluid placed onto the disk at the entry port flows by capillary action to the junction of the each of the metering capillaries comprising the first metering capillary array and the ballast chamber, and to the junction of each of the metering capillaries of the second metering capillary array and the capillary junction, and excess fluid flows by capillary action to the junction of the overflow capillary and the overflow chamber;

and wherein rotation of the platform at a first rotation speed motivates fluid displacement in the overflow capillary into the overflow chamber but not fluid displacement in any of the metering capillaries comprising the first or second metering capillary arrays, whereby rotation of the platform at the first rotational speed drains the fluid from the entry port into the overflow chamber; and

wherein rotation of the platform at a second rotation speed that is greater than the first rotational speed motivates fluid displacement of the volume of the fluid in the metering capillaries of the first capillary array into the ballast chamber; and displacement of the volume of the fluid in the metering capillaries of the second capillary array through the capillary boundary and the channel and into the separation chamber;

wherein each of the ballast chamber, the overflow chamber and the separation chamber also comprise air displacement channels whereby air displaced by fluid movement is

vented to the surface of the platform.

38. The microsystem platform of Claim 37 further comprising
- j) a decant channel having a cross-sectional diameter of about 0.5mm to about 1mm, wherein the channel extends radially from the center of the platform and having a first end proximally arrayed towards the center of the platform and fluidly connected to the separation chamber at a position on the platform that is substantially more proximal to the first end of the chamber than the second end of the chamber, wherein the second end of the decant capillary is fluidly connected with
  - k) a decant chamber having a depth in the surface of the platform equal to or greater than the capillary and positioned radially more distant from the center of the platform than the separation chamber;

wherein the volume of the fluid in the separation chamber delivered from the second metering capillary array is insufficient to fill the separation chamber to a level equal to the position on the platform of the fluid connection to the decant channel;

wherein the decant chamber also comprises air displacement channels whereby air displaced by fluid movement is vented to the surface of the platform.

39. The microsystem platform of Claim 38 further comprising
- l) a capillary having a first end fluidly connected to the ballast chamber and having a second end fluidly connected to a capillary junction having a depth in the surface of the platform equal to or greater than the capillary and positioned radially more distant from the center of the platform than the ballast chamber, and wherein the capillary junction is fluidly connected with
  - m) a channel having a cross-sectional diameter of about 0.5mm to about 1mm, wherein the channel extends radially from the center of the platform and having a first end proximally arrayed towards the center of the platform and fluidly connected to the capillary junction, and a second end that is fluidly connected to the separation chamber at a position equal to the position the second end of the separation chamber,

wherein rotation of the platform at a third rotational speed that is greater than the second

rotational speed motivates fluid displacement of the volume of the fluid in the ballast chamber through the capillary, the capillary junction and the channel and into the separation chamber.

40. The microsystem platform of Claim 39, further comprising
  - n) a sacrificial valve in the capillary extending from the ballast chamber to the separation chamber, wherein release of the sacrificial valve permits fluid flow from the ballast chamber to the separation chamber at a non-zero rotational speed.
41. The microsystem platform of Claim 40 wherein the sacrificial valve is a solid, semi-solid or viscous liquid hydrocarbon, or a plastic.
42. The microsystem platform of Claim 41 further comprising a heating element in the platform in thermal contact with the sacrificial valve, wherein heating the heating element releases the sacrificial valve.
43. A method for separating a fluid from a suspension of particulate in said fluid using a microsystem platform according to Claim 37, the method comprising the steps of
  - a) applying an amount of a fluid sample comprising a particulate suspension having a volume of about 1 to about 50 $\mu$ L to the entry port of the rotatable microsystem platform and allowing the fluid to fill the capillaries of each of the first and second metering capillary arrays and the overflow capillary;
  - b) rotating the platform at a first rotation speed for a time sufficient to displace the fluid in the entry port and the overflow capillary into the overflow chamber;
  - c) rotating the platform at a second rotation speed that is greater than the first rotational speed to displace a volume of the fluid in the first metering capillary into the ballast chamber, and to displace a volume of the fluid from the second metering capillary into the separation chamber;

and

- d) rotating the platform at a third rotation speed that is greater than the first rotational speed for a time sufficient to cause the particulates in the suspension to be concentrated in a portion of the separation chamber that is closer to the second end of the chamber than the first end of the chamber.

44. A method for separating a fluid from a suspension of particulate in said fluid using a microsystem platform according to Claim 39, the method comprising the steps of

- a) applying an amount of a fluid sample comprising a particulate suspension having a volume of about 1 to about 50 $\mu$ L to the entry port of the rotatable microsystem platform and allowing the fluid to fill the capillaries of each of the first and second metering capillary arrays and the overflow capillary;
- b) rotating the platform at a first rotation speed for a time sufficient to displace the fluid in the entry port and the overflow capillary into the overflow chamber;
- c) rotating the platform at a second rotation speed that is greater than the first rotational speed to displace a volume of the fluid in the first metering capillary into the ballast chamber, and to displace a volume of the fluid from the second metering capillary into the separation chamber;
- d) rotating the platform at a third rotation speed that is greater than the second rotational speed for a time sufficient to cause the particulates in the suspension to be concentrated in a portion of the separation chamber that is closer to the second end of the chamber than the first end of the chamber;
- e) rotating the platform at a fourth rotational speed that is greater than the third rotational speed to motivate displacement of fluid from the ballast chamber into the separation column, wherein the level of the fluid in the separation column rises to the level of the fluid connection between the separation column and the decant channel, whereby displacement of the



volume of the fluid in the ballast chamber into the separation chamber decants an equal volume into the decant chamber, wherein the fluid is substantially free of particulates.

45. A method according to Claim 37 wherein the fluid is blood
46. A method according to Claim 38 wherein the fluid is blood
47. A method according to Claim 39 wherein the fluid is blood.
48. A method according to Claim 44 wherein the fluid is blood.
49. A microsystem platform of Claim 39 wherein the particulate suspension has a viscosity that prevents fluid flow through the second capillary.
50. A microsystem platform comprising
  - a) a rotatable platform, comprising a substrate having a first flat, planar surface and a second flat, planar surface opposite thereto, each surface comprising a center about which the platform is rotated, wherein the first surface comprises in combination
  - b) a first fluid chamber comprising a volume of a first fluid and a second fluid chamber comprising a second volume of a second fluid, wherein the first fluid chamber is fluidly connected to a first capillary and the second fluid chamber is fluidly connected to a second capillary, and wherein each of the first and second capillaries are fluidly connected to
  - c) a capillary junction having a depth in the surface of the platform equal to or greater than the first or second capillaries and positioned radially more distant from the center of the platform than either of the fluid chambers, and wherein the capillary junction is fluidly connected to
  - d) a mixing chamber inlet capillary extending radially on the platform from the capillary junction and being further fluidly connected to
  - e) a mixing chamber having a depth in the surface of the platform equal to or greater than the inlet capillary and positioned radially more distant from the center of the platform than either of the capillary junction, the mixing chamber further comprising a mixing chamber outlet capillary

fluidly connected thereto.

51. A microsystem platform according to Claim 50 wherein the volumes contained in the first and second fluid chambers are equal.

52. A microsystem platform according to Claim 50 wherein the volumes contained in the first and second fluid chambers are unequal.

53. A microsystem platform according to Claim 50 wherein the volumes contained in the first and second fluid chambers are mixed to produce a gradient, wherein the shape and position of the first fluid chamber produces a greater fluid volume than the fluid volume displaced from the second fluid chamber when the platform is first rotated at a non-zero rotational speed, and wherein said displaced fluid volume in the first fluid chamber is reduced at a faster rate during rotation than the displaced fluid volume from the second fluid chamber.

54. A microsystems platform according to Claim 50 further comprising  
e) a mixed fluid receiving chamber having a depth in the surface of the platform equal to or greater than the capillaries and positioned radially more distant from the center of the platform than the mixing chamber.

55. A microsystems platform according to Claim 54, further comprising a multiplicity of mixing chambers arrayed radially across the platform surface, each mixing chamber having an inlet capillary extending radially from a position more proximal to the center of rotation than the mixing chamber, and an outlet capillary extending radially to a position more distal to the center of rotation than the mixing chamber, wherein the inlet capillary of each mixing chamber is fluidly connected with the first mixing chamber of Claim 50 or is the outlet chamber of the mixing chamber immediately more proximal to the center of rotation, and the outlet capillary of each mixing chamber is the inlet capillary of the mixing chamber immediately more distal to the center of rotation, and wherein the outlet capillary of the mixing chamber positioned the most distal from the center of rotation is fluidly connected with a mixed fluid

receiving chamber.

56. A microsystems platform according to Claim 53 further comprising a mixed fluid receiving chamber having a depth in the surface of the platform equal to or greater than the capillaries and positioned radially more distant from the center of the platform than the mixing chamber, wherein the mixing chamber comprises a multiplicity of compartments laterally arrayed on the platform, wherein the outlet capillary is fluidically attached to the mixed fluid receiving chamber at a position most proximal to the center of rotation and at the lateral side of the mixing chamber, wherein fluid flow through the capillary and into the mixing chamber sequentially fills each of the compartments of the mixing chamber from the compartment at the closest lateral extent to the fluid connection to the outlet capillary to the compartment at the farthest lateral extent to the fluid connection to the outlet capillary.

57. A method of mixing two fluids using a microsystem platform according to Claim 54, the method comprising the steps of:

- a) applying a volume of a first fluid to the first fluid chamber and applying a volume of a second fluid to the second fluid chamber;
- b) rotating the platform at a rotational speed sufficient to motivate fluid flow from the fluid chambers through the capillaries and past the capillary junction and into the mixing chamber, whereby turbulent fluid flow is produced in the mixing chamber; and
- c) collecting the mixed fluids in the mixed fluid receiving chamber.

58. The method of claim 57 wherein the two fluids differ in the concentration of a solute.

59. The method of claim 57 wherein the two fluids have different viscosities.

60. A microsystem platform comprising

- a) a rotatable platform, comprising a substrate having a first flat, planar surface and a second flat, planar surface opposite thereto, each surface

comprising a center about which the platform is rotated, wherein the first surface comprises in combination

- b) a first entry port and a second entry port, each comprising a depression in the first surface having a volumetric capacity of about 5 to about 50 $\mu$ L, that is fluidly connected with
- c) a first capillary array and a second capillary array, each comprising a multiplicity of capillaries, each capillary of the first capillary array being fluidly connected with the first entry port, and each capillary of the second capillary array being fluidly connected with the second entry port, wherein each capillary defines a cross-sectional area of about 0.02mm to about 1mm in diameter, and wherein each capillary extends radially from the center of the platform and defines a first end proximally arrayed towards the center of the platform and a second end distally arrayed from the center of the platform, wherein the proximal end of each capillary defines a curved opening; wherein the capillary array defines a volume of the fluid

and wherein the capillary arrays are fluidly connected with

- d) a curved capillary barrier having a depth in the surface of the platform equal to or greater than the capillary and positioned radially more distant from the center of the platform, wherein a capillary junction is formed at the junction of each of the capillaries comprising the capillary arrays and the curved capillary barrier;

whereby a first fluid placed onto the platform at the first entry port flows by capillary action to the junction of the each of the capillaries comprising the first capillary array and the curved capillary junction, and a second fluid placed onto the platform at the second entry port flows by capillary action to the junction of the each of the capillaries comprising the second capillary array and the curved capillary junction,

and wherein rotation of the platform at a first rotation speed motivates fluid displacement of the volume of the fluid in the capillaries of the first and second capillary arrays into the curved capillary junction; and wherein

the curved capillary junction also comprise air displacement channels whereby air displaced by fluid movement is vented to the surface of the platform.

61. A microsystems platform according to Claim 60 further comprising:
- e) a mixing chamber inlet capillary extending radially on the platform from the curved capillary junction and being further fluidly connected to
  - f) a mixing chamber having a depth in the surface of the platform equal to or greater than the inlet capillary and positioned radially more distant from the center of the platform than the capillary junction, the mixing chamber further comprising a mixing chamber outlet capillary fluidly connected thereto.
62. A microsystems platform according to Claim 61 further comprising:
- g) a mixed fluid receiving chamber having a depth in the surface of the platform equal to or greater than the mixing chamber outlet capillary and positioned radially more distant from the center of the platform than the mixing chamber.
63. A microsystem platform according to Claim 60 wherein the volumes contained in the first and second fluid chambers are equal.
64. A microsystem platform according to Claim 60 wherein the volumes contained in the first and second fluid chambers are unequal.
65. A microsystems platform according to Claim 62, further comprising a multiplicity of mixing chambers arrayed radially across the platform surface, each mixing chamber having an inlet capillary extending radially from a position more proximal to the center of rotation than the mixing chamber, and an outlet capillary extending radially to a position more distal to the center of rotation than the mixing chamber, wherein the inlet capillary of each mixing chamber is fluidly connected with the first mixing chamber of Claim 62 or is the outlet chamber of the mixing chamber immediately more proximal to the center of rotation, and the outlet capillary of each mixing chamber is the inlet capillary of the mixing chamber immediately more distal to the center of rotation, and wherein the outlet capillary of the mixing chamber positioned

the most distal from the center of rotation is fluidly connected with a mixed fluid receiving chamber.

66. A microsystems platform according to Claim 61 further comprising a mixed fluid receiving chamber having a depth in the surface of the platform equal to or greater than the capillaries and positioned radially more distant from the center of the platform than the mixing chamber, wherein the mixing chamber comprises a multiplicity of compartments laterally arrayed on the platform, wherein the outlet capillary is fluidically attached to the mixed fluid receiving chamber at a position most proximal to the center of rotation and at the lateral side of the mixing chamber, wherein fluid flow through the capillary and into the mixing chamber sequentially fills each of the compartments of the mixing chamber from the compartment at the closest lateral extent to the fluid connection to the outlet capillary to the compartment at the farthest lateral extent to the fluid connection to the outlet capillary.

68. A method of mixing two fluids using a microsystem platform according to Claim 62, the method comprising the steps of:

- a) applying a volume of a first fluid to a first entry port and applying a volume of a second fluid to the second entry port and allowing the fluid to fill the first and second capillary arrays by capillary action;
- b) rotating the platform at a first rotational speed sufficient to motivate fluid flow from the capillary arrays into the curved capillary junction;
- c) rotating the platform at a second rotational speed that is greater than the first rotational speed to motivate fluid flow past the capillary junction, through the mixing chamber inlet capillary and into the mixing chamber, whereby turbulent fluid flow is produced in the mixing chamber; and
- d) collecting the mixed fluids in the mixed fluid receiving chamber.

69. The method of claim 68 wherein the two fluids differ in the concentration of a solute.

70. The method of claim 68 wherein the two fluids have different viscosities.

71. A microsystem platform comprising
- a) a rotatable platform, comprising a substrate having a first flat, planar surface and a second flat, planar surface opposite thereto, each surface comprising a center about which the platform is rotated, wherein the first surface comprises in combination
  - b) an entry port comprising a depression in the first surface having a volumetric capacity of about 1 to about 100 $\mu$ L, that is fluidly connected with
  - c) a metering capillary and an overflow capillary, each being fluidly connected with the entry port, wherein each capillary defines a cross-sectional area of about 0.02mm to about 1mm in diameter, and wherein each capillary extends radially from the center of the platform and defines a first end proximally arrayed towards the center of the platform and a second end distally arrayed from the center of the platform, wherein the proximal end of each capillary defines a curved opening; wherein the metering capillary defines a volume of the fluid and wherein the metering capillary is fluidly connected with
  - d) a first capillary junction having a depth in the surface of the platform equal to or greater than the metering capillary and positioned radially more distant from the center of the platform than the entry port, and wherein the overflow capillary is fluidly connected with
  - e) an overflow chamber having a depth in the surface of the platform equal to or greater than the overflow capillary and positioned radially more distant from the center of the platform than the holding channel and the entry port,

wherein a capillary junction is formed at the junction of the metering capillary and the first capillary junction, and at the junction of the overflow capillary and the overflow chamber, whereby fluid placed onto the disk at the entry port flows by capillary action to the junction of the metering capillary and the first capillary junction, and excess fluid flows by capillary action to the junction of the overflow capillary and the overflow chamber; and wherein rotation of the platform at a first rotation speed motivates fluid displacement in the overflow capillary into the overflow chamber but not fluid

displacement in the metering capillary, whereby rotation of the platform at the first rotational speed drains the fluid from the entry port into the overflow chamber; and wherein rotation of the platform at a second rotation speed that is greater than the first rotational speed motivates fluid displacement of the volume of the fluid in the metering capillary past the first capillary junction; and wherein the overflow chamber also comprises air displacement channels whereby air displaced by fluid movement is vented to the surface of the platform.

71. The microsystem platform of Claim 70 further comprising
6. an incubation chamber inlet capillary having a first end fluidly connected to the first capillary junction and having a second end fluidly connected to
7. an incubation chamber, having a depth in the surface of the platform equal to or greater than the capillary and positioned radially more distant from the center of the platform than the first capillary junction

whereby fluid in the metering capillary flows past the first capillary junction and through the incubation chamber inlet capillary into the incubation chamber when the platform is rotated at a second rotational speed.

72. The microsystem platform of Claim 71 further comprising
- f) an incubation chamber outlet capillary having a first end fluidly connected to the incubation chamber at a position most distal from the center of rotation, and having a second end fluidly connected to
- g) an waste chamber, having a depth in the surface of the platform equal to or greater than the capillary and positioned radially more distant from the center of the platform than the incubation chamber

wherein the incubation chamber outlet position extends radially from the position of fluid connection with the incubation chamber substantially parallel to the lateral extent of the incubation chamber, and wherein the capillary comprises a substantially semicircular bend at a position about equal to the side of the incubation chamber most proximal to the center of rotation to produce a pressure block, whereby fluid flow in the outlet capillary is balanced by the volume of the fluid in the incubation chamber, thereby



preventing fluid flow into the waste chamber at the second rotational speed.

73. The microsystem platform of Claim 72 further comprising
- h) a wash buffer reservoir positioned radially more distant from the center of the platform than the entry port but less distant from the center of rotation than the incubation chamber, wherein the wash buffer reservoir is fluidly connected with
  - l) a wash buffer outlet capillary having a first end fluidly connected to the wash buffer reservoir and having a second end fluidly connected to
  - j) a second capillary junction having a depth in the surface of the platform equal to or greater than the outlet capillary and positioned radially more distant from the center of the platform than the wash buffer reservoir, wherein the capillary junction is fluidly connected to the incubation chamber inlet capillary

wherein rotation of the platform at a third rotational speed that is greater than the second rotational speed motivates fluid flow of the wash buffer through the wash buffer outlet capillary, the capillary junction and the incubation inlet capillary and into the incubation chamber, whereby displacement of the volume of the fluid in the incubation chamber by the wash buffer overcomes the pressure block in the incubation chamber outlet capillary, thereby permitting the fluid in the incubation chamber to be displaced by the wash buffer and to flow into the waste chamber at the third rotational speed.

74. The microsystem platform of Claim 73 further comprising
- k) a reagent reservoir positioned radially less distant from the center of the platform than the incubation chamber, wherein the reagent reservoir is fluidly connected with
  - l) a reagent outlet capillary having a first end fluidly connected to the reagent reservoir and having a second end fluidly connected to
  - m) the second capillary junction,

wherein rotation of the platform at a fourth rotational speed that is greater than the third rotational speed motivates fluid flow of the wash buffer through the reagent reservoir outlet capillary, the capillary junction and the incubation inlet capillary and into the

incubation chamber, whereby displacement of the volume of the wash buffer in the incubation chamber by the reagent overcomes the pressure block in the incubation chamber outlet capillary, thereby permitting the fluid in the incubation chamber to be displaced by the reagent and to flow into the waste chamber at the fourth rotational speed.

75. The microsystem platform of Claim 74, further comprising
- a) a sacrificial valve in the capillary extending from the reagent reservoir to the incubation chamber, wherein release of the sacrificial valve permits fluid flow from the holding chamber to the read chamber at a non-zero rotational speed.

76. The microsystem platform of Claim 75 wherein the sacrificial valve is a solid, semi-solid or viscous liquid hydrocarbon, or a plastic.

77. The microsystem platform of Claim 76 further comprising a heating element in the platform in thermal contact with the sacrificial valve, wherein heating the heating element releases the sacrificial valve.

78. A method for performing an affinity binding assay using a microsystem platform according to Claim 72, the method comprising the steps of

- a) applying an amount of a fluid sample comprising a first member of an affinity binding pair and having a volume of about 1 to about 100 $\mu$ L to the entry port of the rotatable microsystem platform and allowing the metering capillary and overflow capillary to fill by capillary action;
- b) rotating the platform at a first rotation speed for a time sufficient to displace the fluid in the entry port and the overflow capillary into the overflow chamber;
- c) rotating the platform at a second rotation speed that is greater than the first rotational speed displace a volume of the fluid in the metering capillary into the incubation chamber, wherein the incubation chamber contains a second member of the affinity binding pair; and

- d) detecting affinity binding in the incubation chamber.

79. A method for performing an affinity binding assay using a microsystem platform according to Claim 73, the method comprising the steps of

- a) applying an amount of a fluid sample comprising a first member of an affinity binding pair and having a volume of about 1 to about 100 $\mu$ L to the entry port of the rotatable microsystem platform and allowing the metering capillary and overflow capillary to fill by capillary action;
- b) rotating the platform at a first rotation speed for a time sufficient to displace the fluid in the entry port and the overflow capillary into the overflow chamber;
- c) rotating the platform at a second rotational speed that is greater than the first rotational speed displace a volume of the fluid in the metering capillary into the incubation chamber, wherein the incubation chamber contains a second member of the affinity binding pair;
- d) incubating the fluid in the incubation chamber for a time sufficient to form the affinity binding pair;
- e) rotating the platform at a third rotational speed that is greater than the second rotational speed to displace a volume of the wash fluid into the incubation chamber; and
- f) detecting affinity binding in the incubation chamber.

80. A method for performing an affinity binding assay using a microsystem platform according to Claim 74, the method comprising the steps of

- a) applying an amount of a fluid sample comprising a first member of an affinity binding pair and having a volume of about 1 to about 100 $\mu$ L to the entry port of the rotatable microsystem platform and allowing the metering capillary and overflow capillary to fill by capillary action;
- b) rotating the platform at a first rotation speed for a time sufficient to displace the fluid in the entry port and the overflow capillary into the overflow chamber;

- c) rotating the platform at a second rotational speed that is greater than the first rotational speed to displace a volume of the fluid in the metering capillary into the incubation chamber, wherein the incubation chamber contains a second member of the affinity binding pair;
- d) incubating the fluid in the incubation chamber for a time sufficient to form the affinity binding pair;
- e) rotating the platform at a third rotational speed that is greater than the second rotational speed to displace a volume of the wash fluid into the incubation chamber;
- f) rotating the platform at a fourth rotational speed that is greater than the third rotational speed to displace a volume of the reagent into the incubation chamber;
- g) incubating the reagent in the incubation chamber for a time sufficient to produce an amount of a detectable product that is proportional to the amount of affinity binding; and
- h) detecting affinity binding in the incubation chamber.

81. A resistive heater element comprising in combination:

- a). an electrically inert substrate capable of being screen printed with a conductive ink and a resistive ink;
- b) a conductive ink screen-printed in a pattern;
- c) a resistive ink screen-printed in a pattern over the conductive ink pattern wherein the resistive ink is in electrical contact with the conductive ink and wherein an electrical potential applied across the conductive ink causes current to flow across the resistive ink wherein the resistive ink produces heat.

82. The resistive heater element according to Claim 81 wherein the conductive ink is a silver conductive ink selected from the group consisting of Dupont 5028, Dupont 5025, Acheson 423SS, Acheson 426SS and Acheson SS24890.

83. The resistive heater element according to Claim 81 wherein the resistive

ink is selected from the group consisting of Dupont 7082, Dupont 7102, Dupont 7271, Dupont 7278 and Dupont 7285

84. A resistive heater element according to Claim 81 wherein the resistive ink is a PTC ink

85. A resistive heater element according to Claim 81 further comprising

d) a dielectric ink screen-printed over the resistive ink pattern and conductive ink pattern.

86. A heat-activated wax valve in a microfluidics array of a microsystems platform comprising, in combination

- a) an amount of a solid, semi-solid or viscous liquid hydrocarbon positioned to occlude the lumen of a channel or capillary;
- b) a wax recrystallization chamber positioned in a channel or capillary in fluid contact therein wherein the recrystallization chamber is positioned radially more distant from the center of the platform than the wax valve;
- c) a resistive heater element screen-printed and in thermal contact with the wax valve and at least a portion of the wax recrystallization chamber, wherein the resistive heater element is in electrical contact with a voltage source;

wherein heating the resistive heater element by applying a voltage to the element produced sufficient heat to melt the wax valve, and wherein rotation of the platform motivates the melted wax valve into the wax recrystallization chamber, and wherein the resistive heater element heats the channel or capillary and a portion of the recrystallization chamber, whereby the melted wax valve does not recrystallize in the channel or capillary and thereby does not occlude the lumen of the channel or capillary.

87. A electronic spindle for a centrifugal rotor comprising

a first electrically non-conductive plate having a first side operatively attached to a mechanical spindle and having a second side opposite thereto and having embedded therein

a multiplicity of electrically conductive posts having a first end that is embedded in the electrically non-conductive plate

an alternating array of electrically conductive and non-conductive plates having the conductive posts passing there through, wherein each of the conductive plates is in electrical contact with one of the conductive posts, and wherein none of the conductive posts is in electrical contact with more than one conductive plate, and whereby the electrically conductive plates are insulated from one another,

an electrically conductive plate having the conductive posts passing there through at an end of the alternating array opposite to the first electrically non-conductive plate, wherein the electronic spindle is mechanically and operatively attached to a motor comprising a centrifugation apparatus, and each of the electrically conductive posts is in electrical contact with an electrical contact on a centripetally motivated microsystems platform, and each of the electrically conductive plates is in electrical contact with an electrically conductive brush that transfers an electrical signal from the brush to the electrically conductive plate and to one of the electrically conductive posts, and thereby transfers the electrical signal to a rotating microsystems platform.

88. A microsystem platform according to Claim 50, wherein the inlet and outlet capillaries are connected with the mixing chamber so that their positions in the mixing chamber are offset from one another, wherein fluid flow from the inlet capillary impinges on a wall of the mixing chamber at a position other than the position occupied by the outlet capillary, and wherein fluid flow from the outlet capillary impinges on a wall of the mixing chamber at a position other than the position occupied by the inlet capillary, thereby producing turbulent flow within the mixing chamber that mixes the fluids.

89. A microsystem platform according to Claim 54, wherein the capillaries are connected with the mixing chamber so that their positions in the mixing chamber are offset from one another, wherein fluid flow from the inlet capillary impinges on a wall of the mixing chamber at a position other than the position occupied by the outlet capillary, thereby producing turbulent flow within the mixing chamber that mixes the

fluids.

90. A microsystem platform according to Claim 60, wherein the inlet and outlet capillaries are connected with the mixing chamber so that their positions in the mixing chamber are offset from one another, wherein fluid flow from the inlet capillary impinges on a wall of the mixing chamber at a position other than the position occupied by the outlet capillary, and wherein fluid flow from the outlet capillary impinges on a wall of the mixing chamber at a position other than the position occupied by the inlet capillary, thereby producing turbulent flow within the mixing chamber that mixes the fluids.

91. A microsystem platform according to Claim 64, wherein the capillaries are connected with the mixing chamber so that their positions in the mixing chamber are offset from one another, wherein fluid flow from the inlet capillary impinges on a wall of the mixing chamber at a position other than the position occupied by the outlet capillary, thereby producing turbulent flow within the mixing chamber that mixes the fluids.

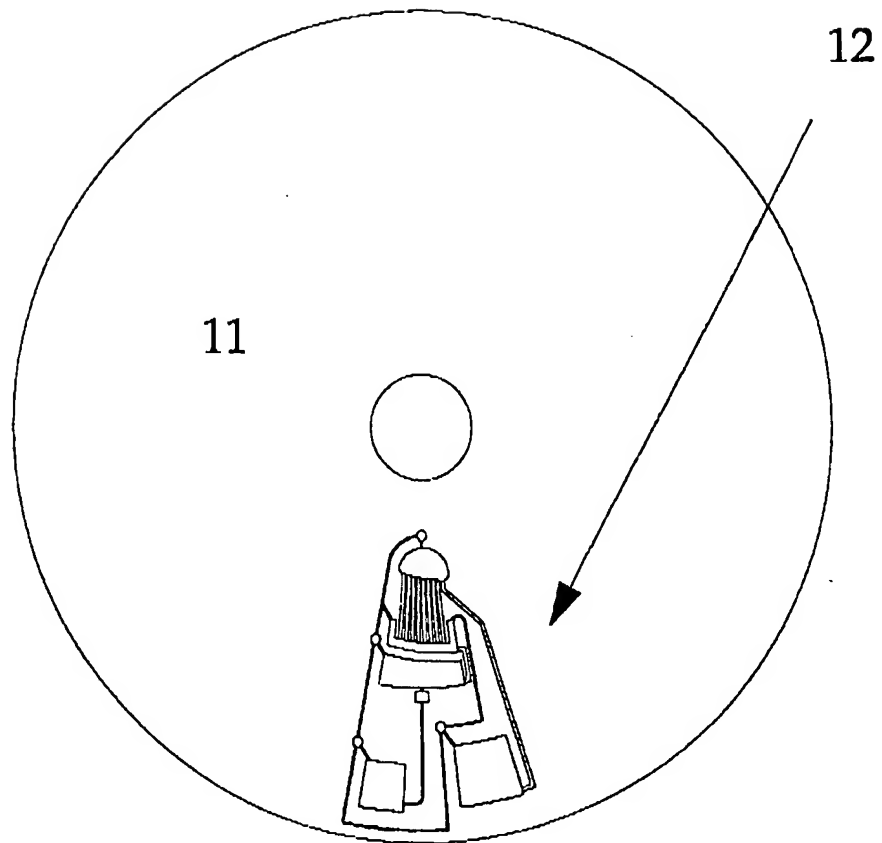


Fig 1



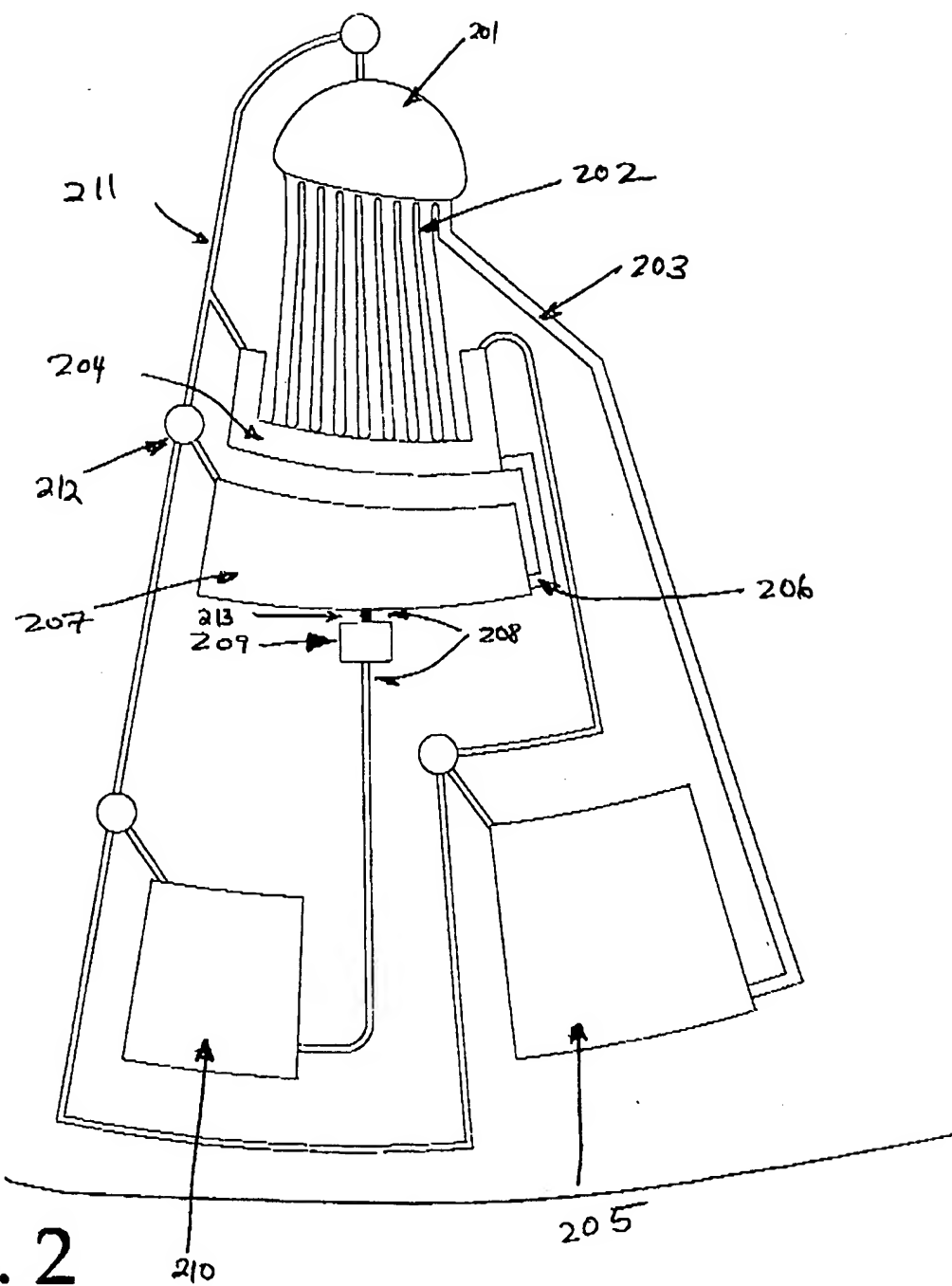
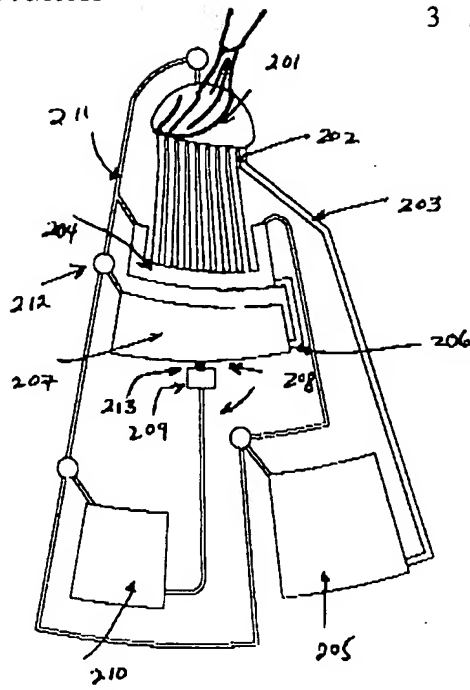
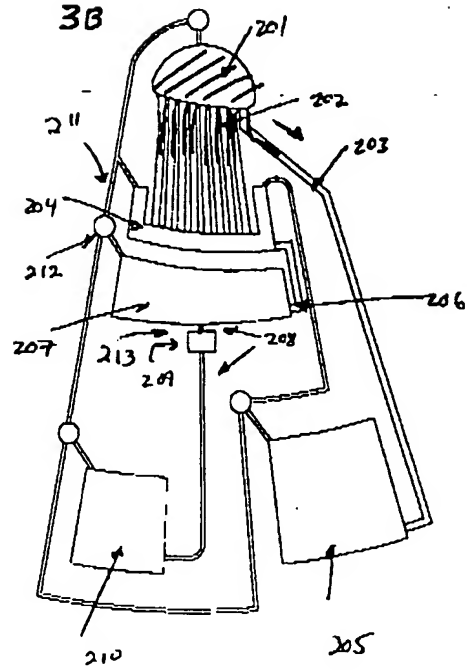


Fig. 2

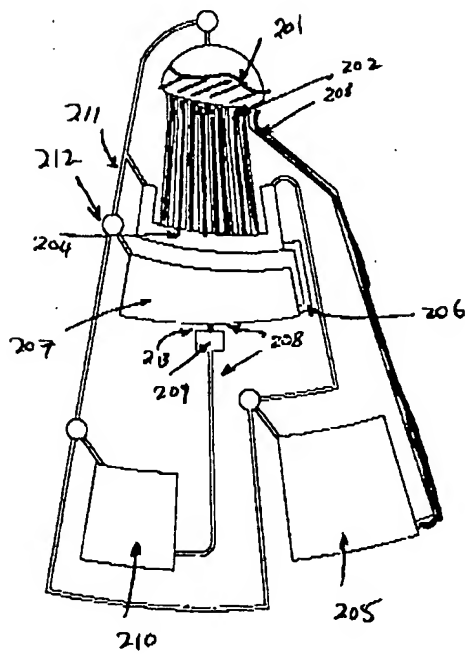
3a



3B



3c



3D

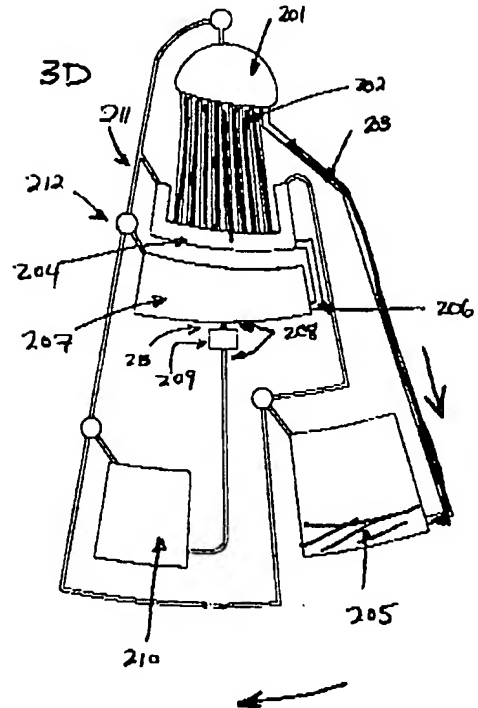


Fig. 3

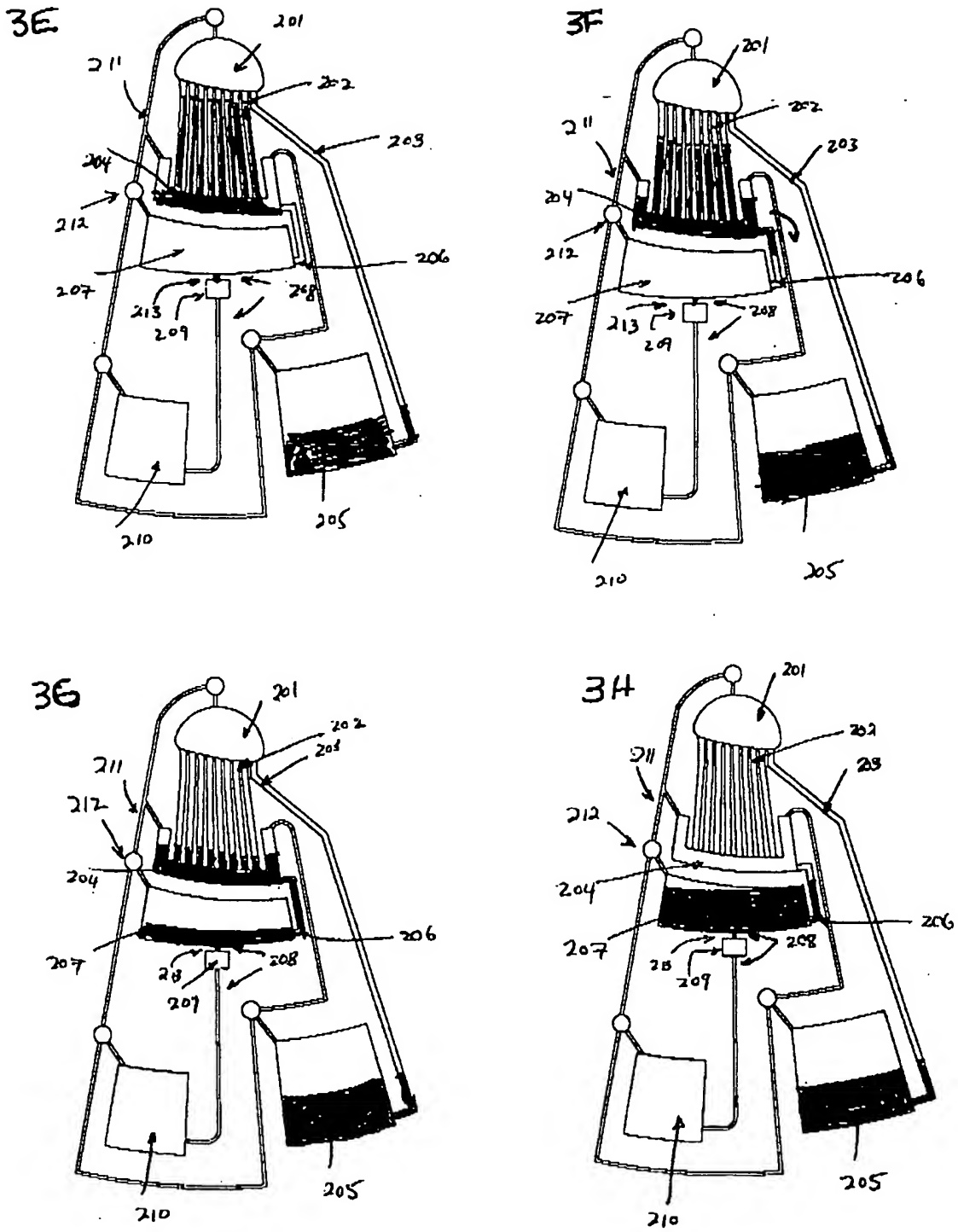


Fig. 3

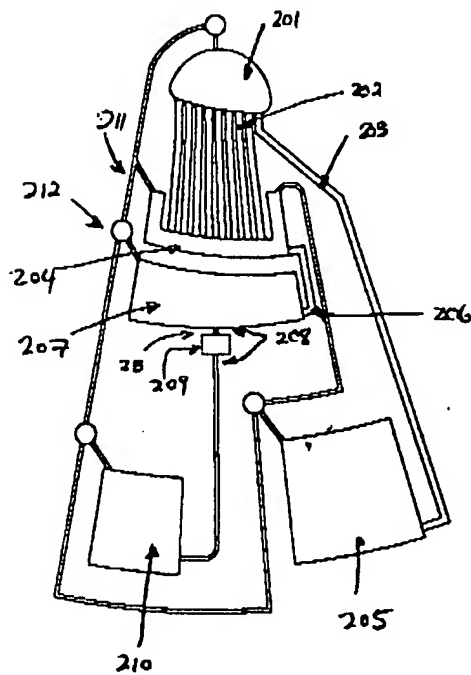
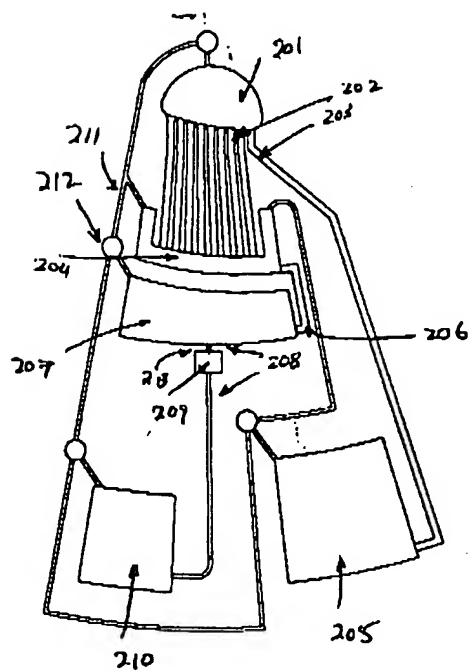
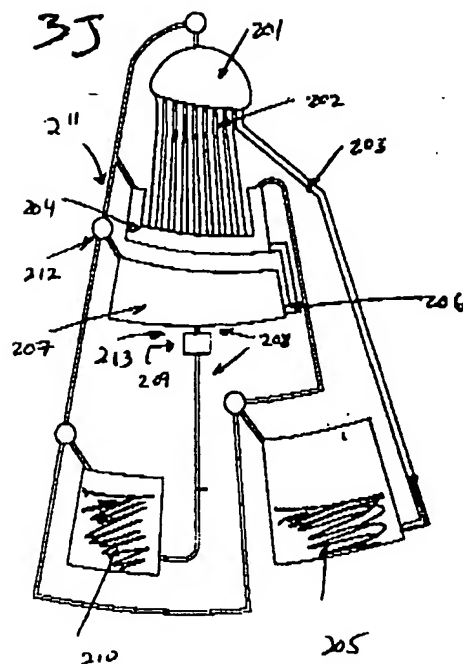
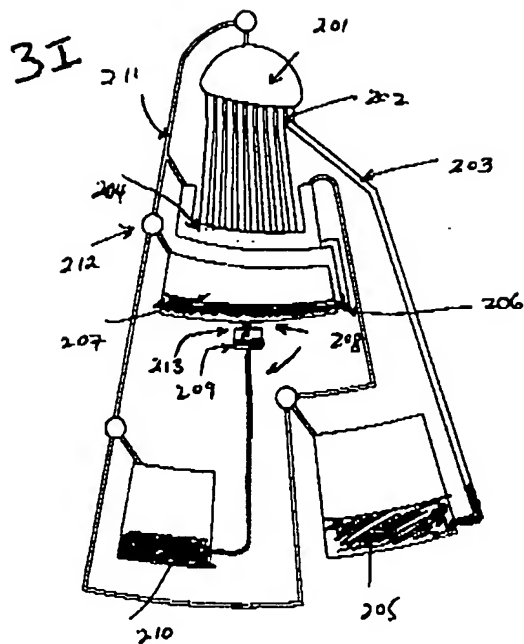


Fig. 3

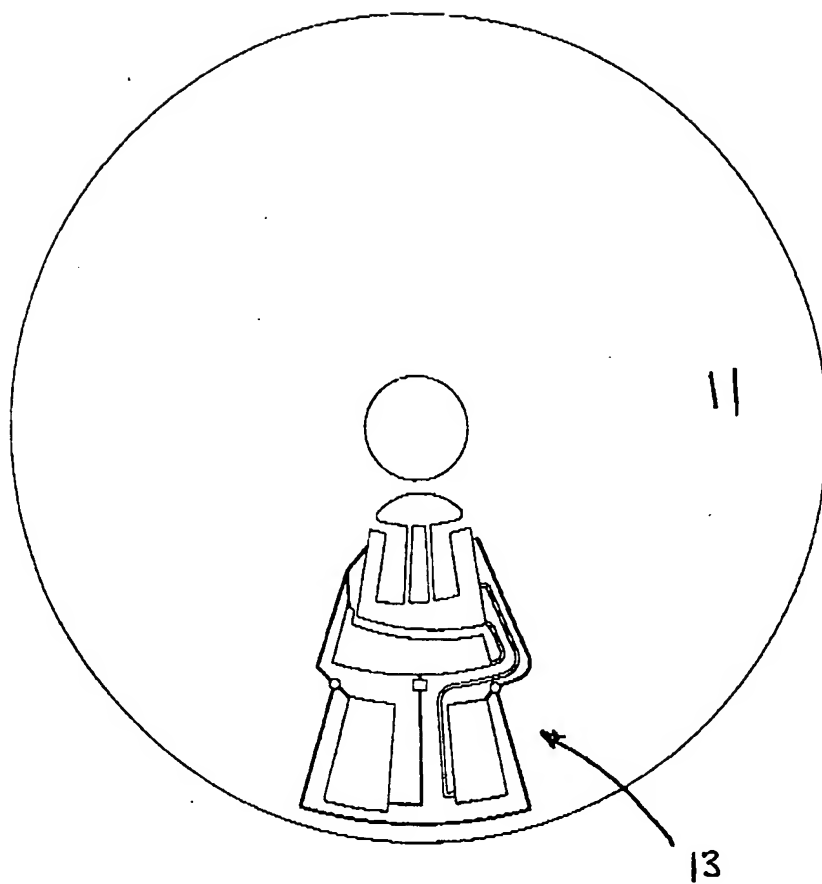


Fig. 4

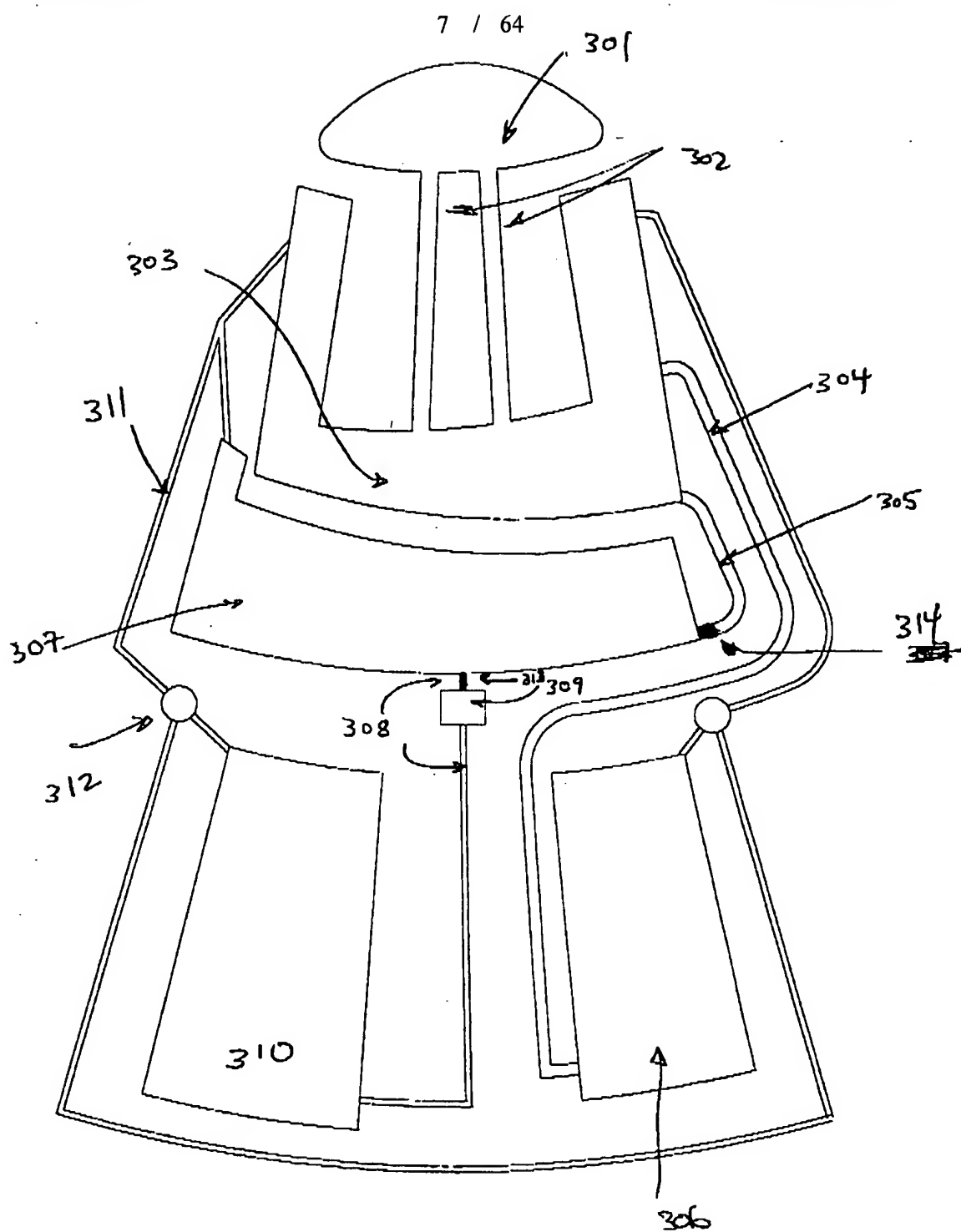


Fig. 5

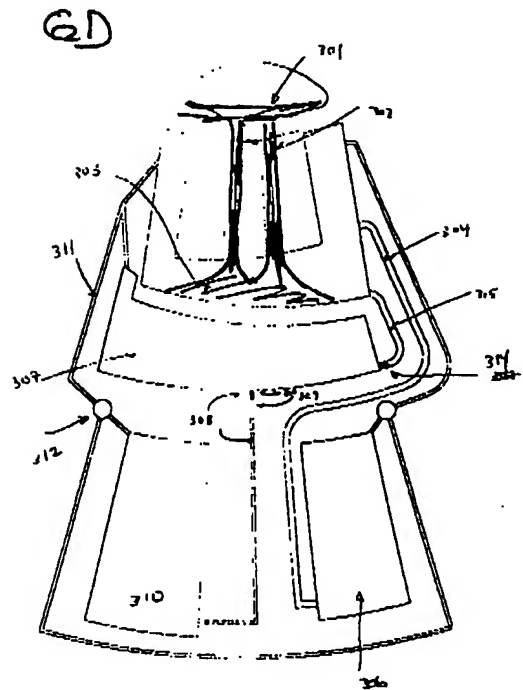
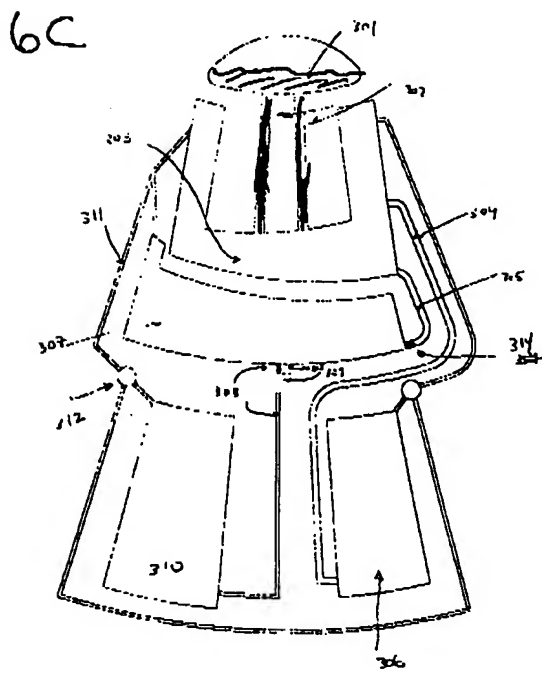
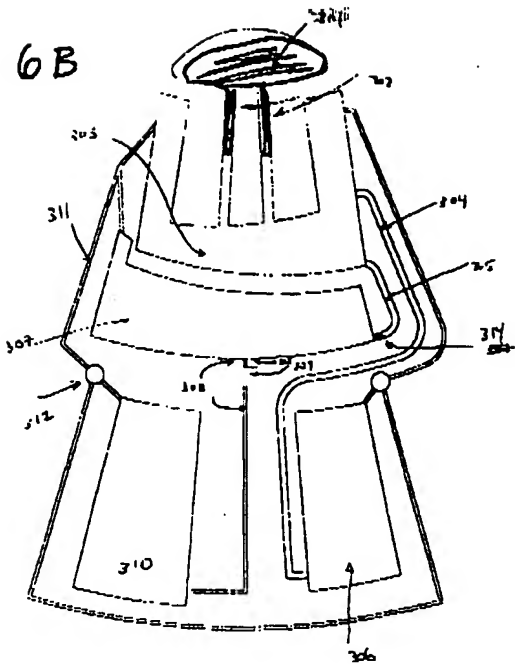
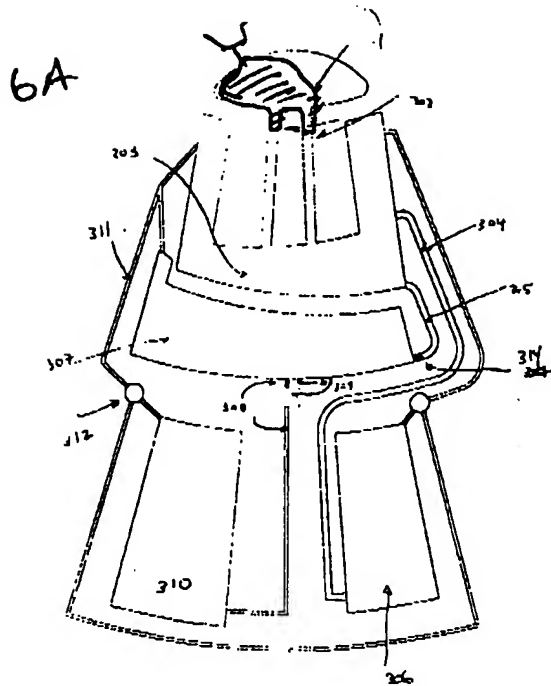
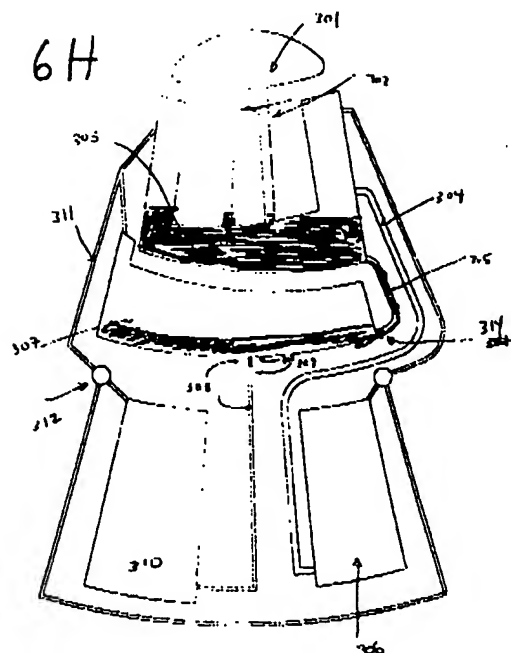
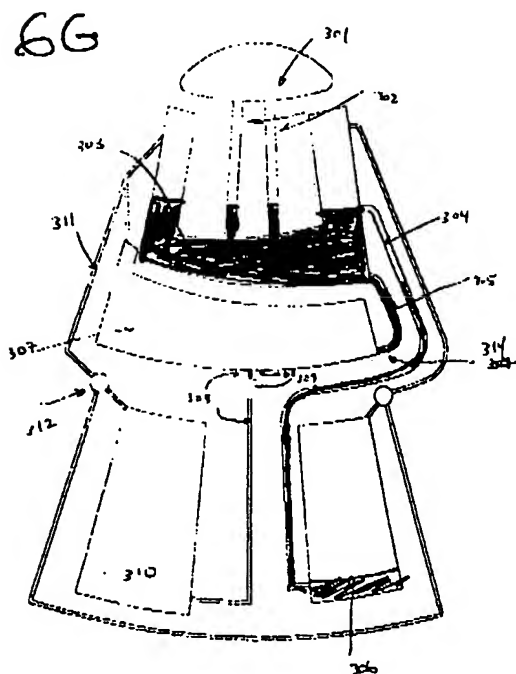
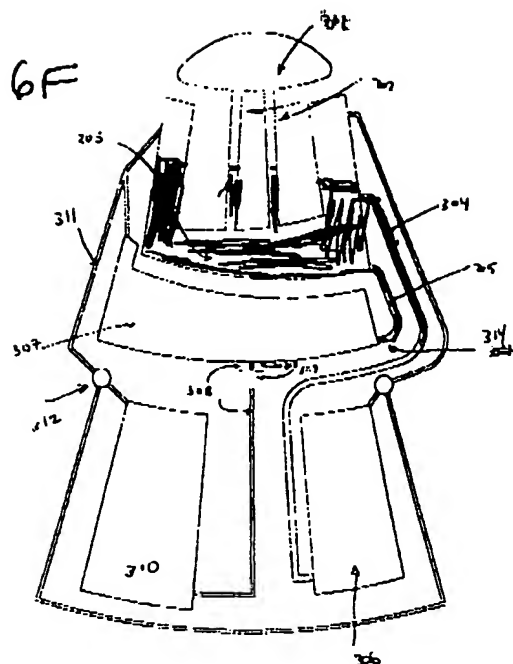
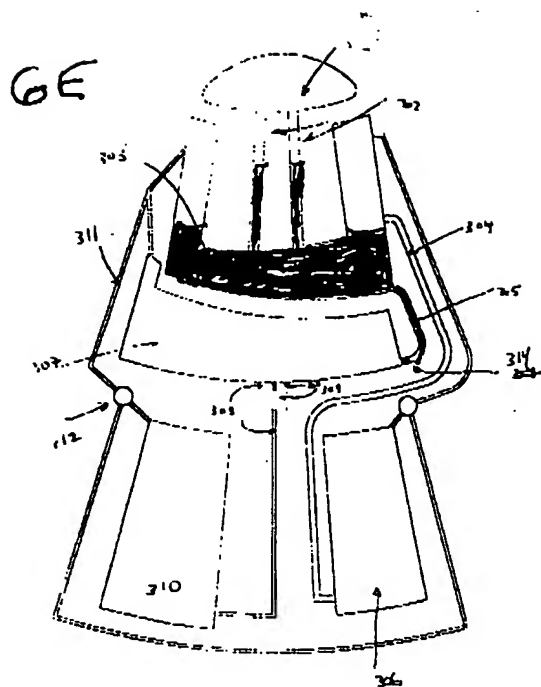
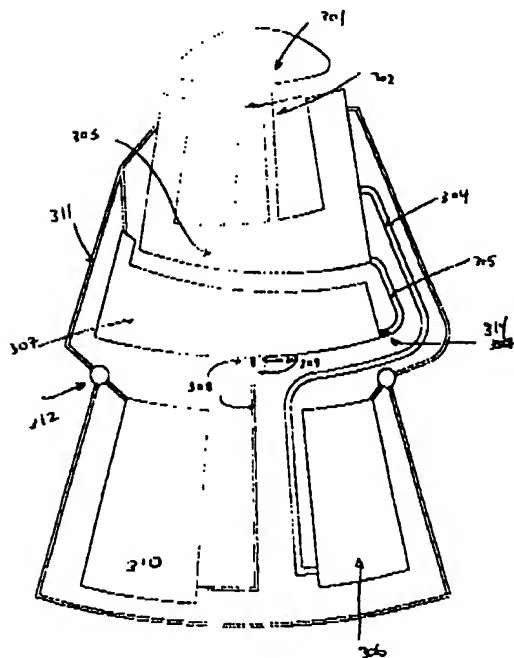
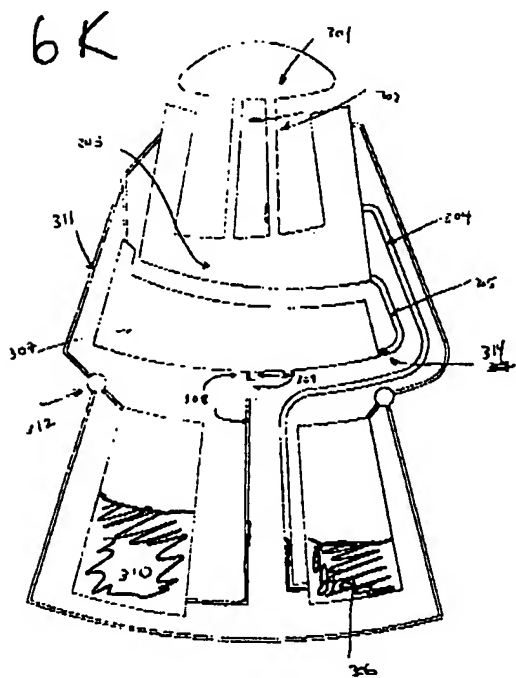
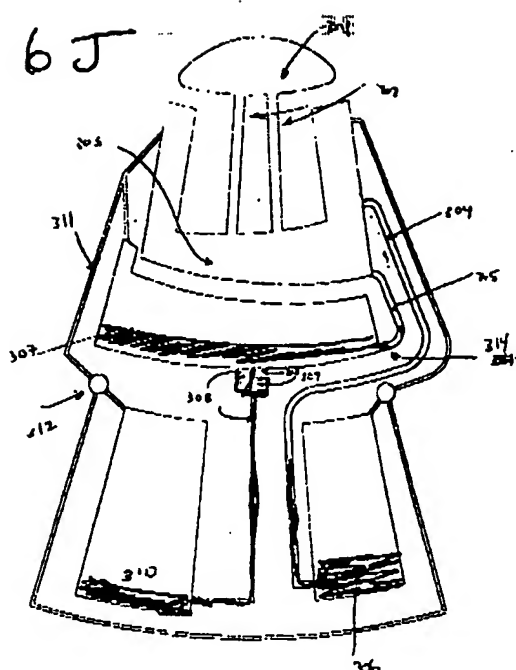
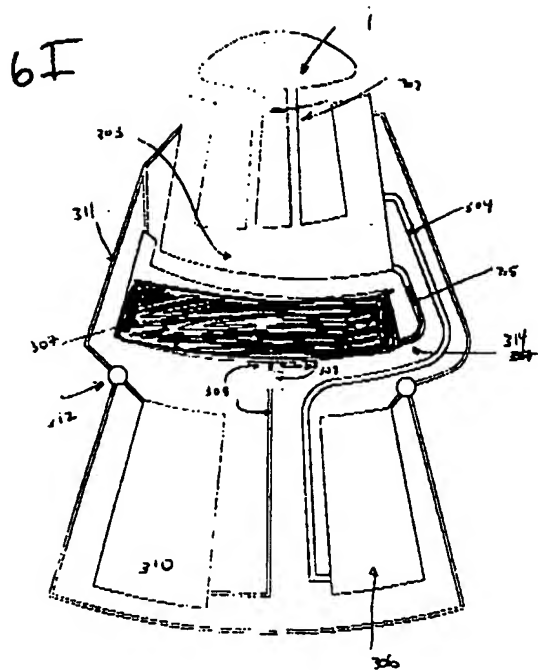


FIG 6







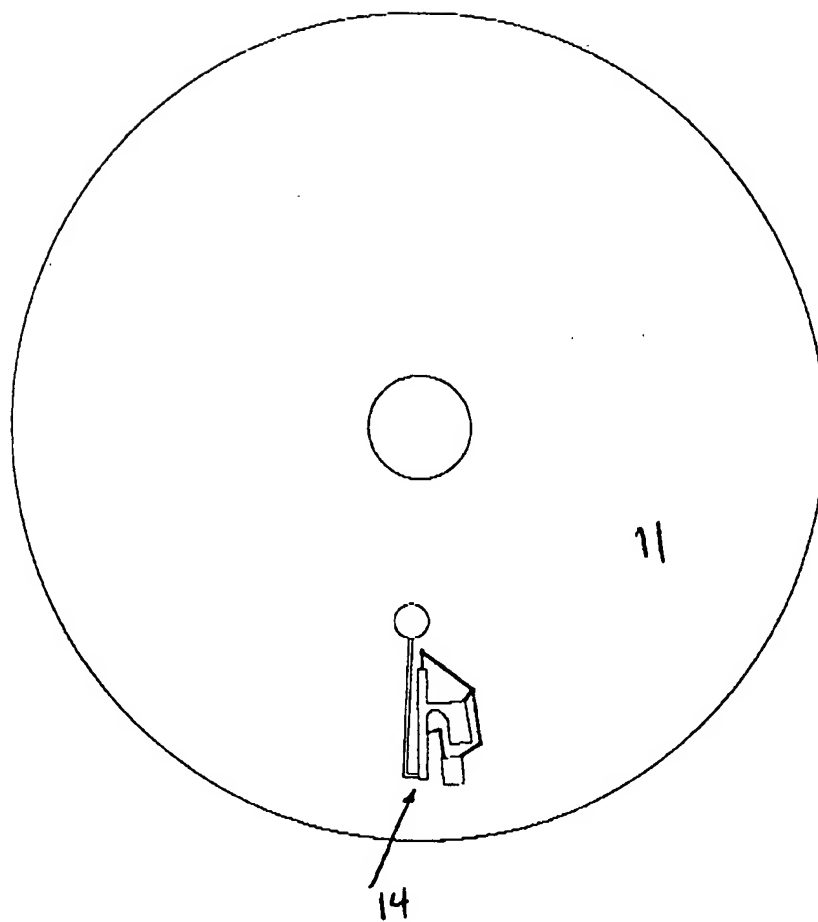


Fig. 7

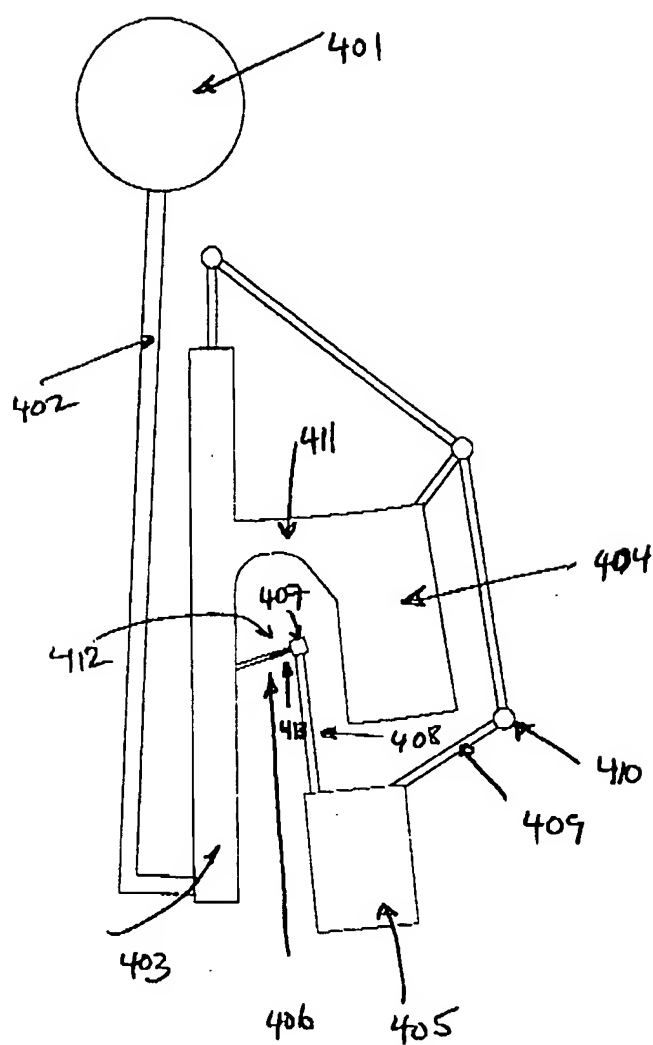


Fig 8

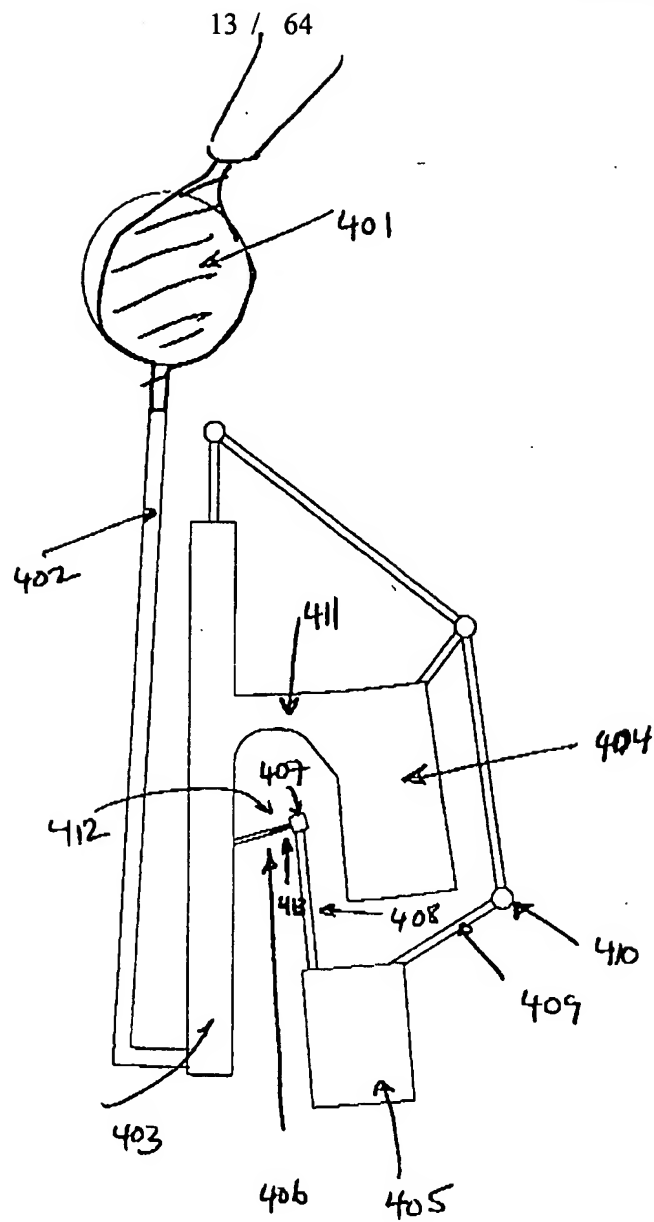


FIG. 9A

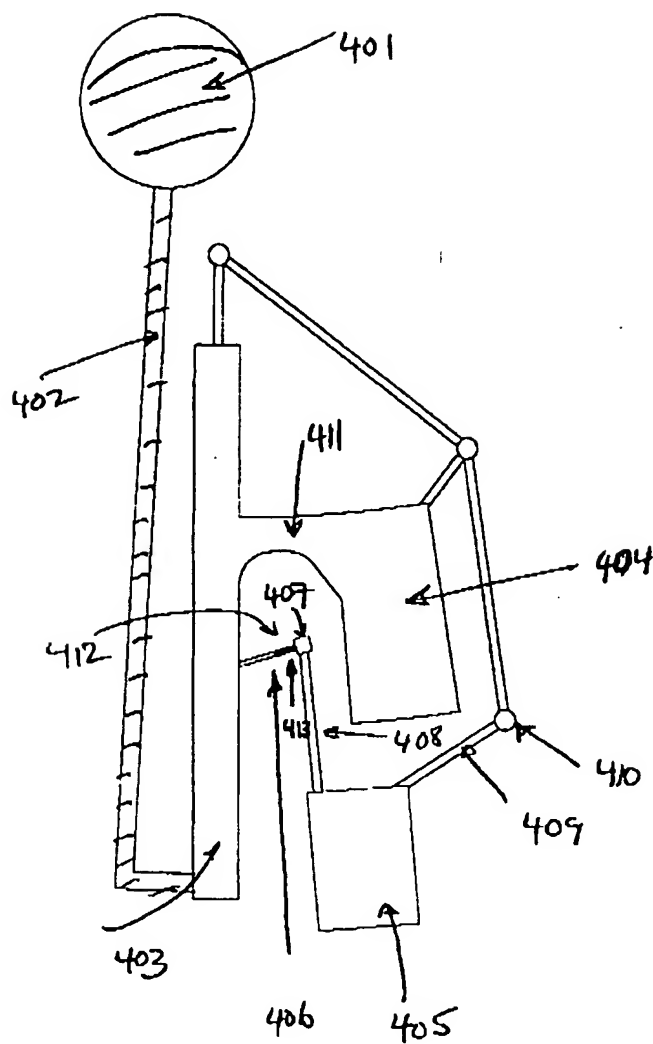


FIG. 9B

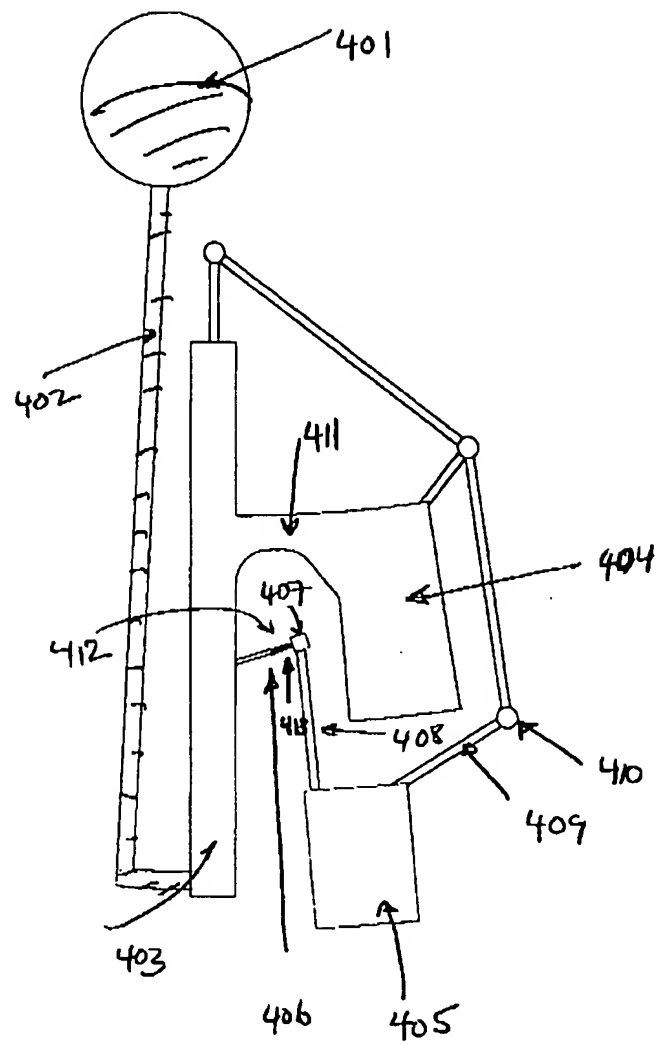


FIG. 9C



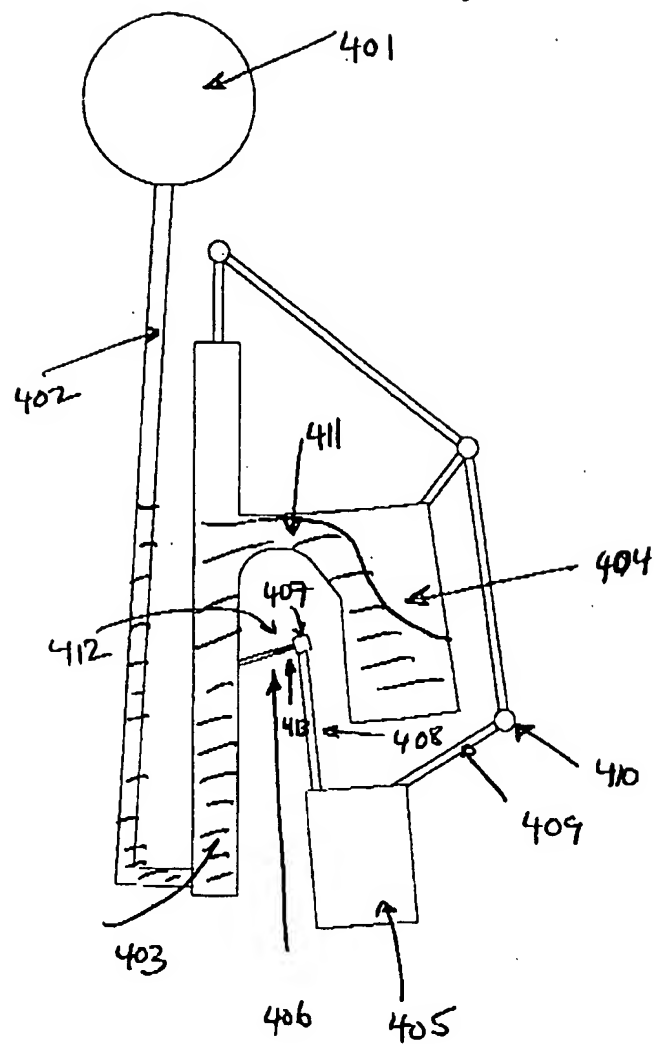


FIG. 9E



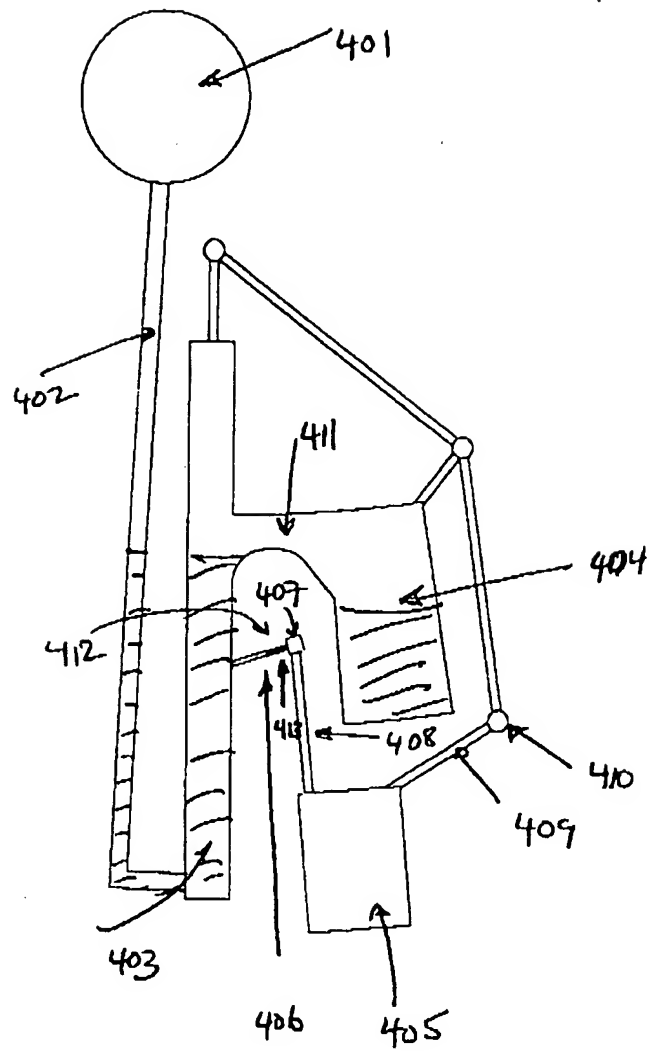


FIG. 9F

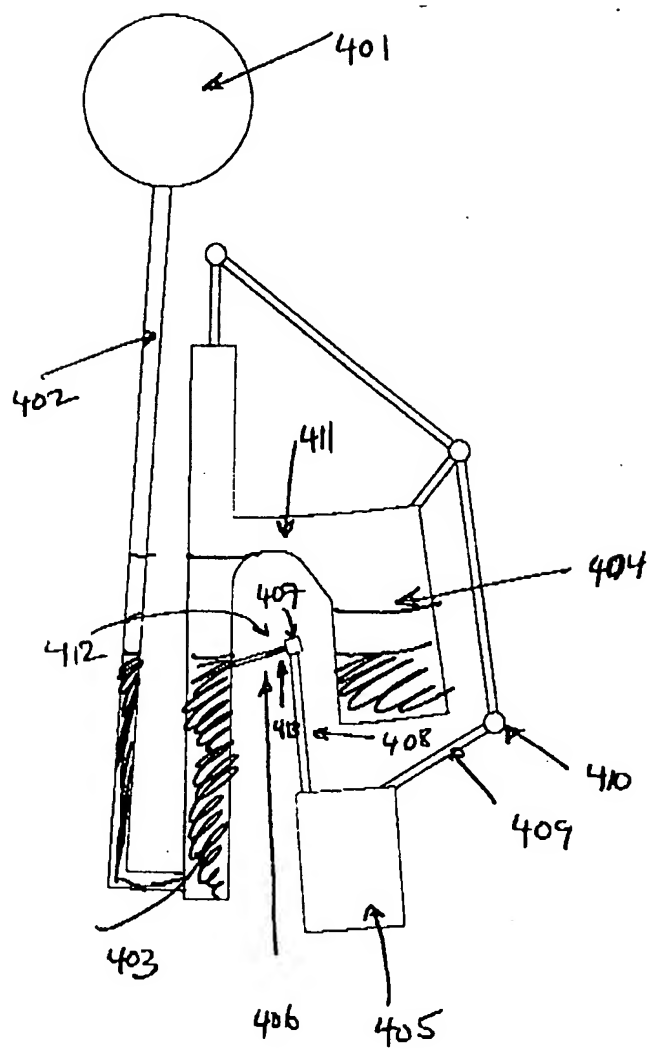


FIG. 96

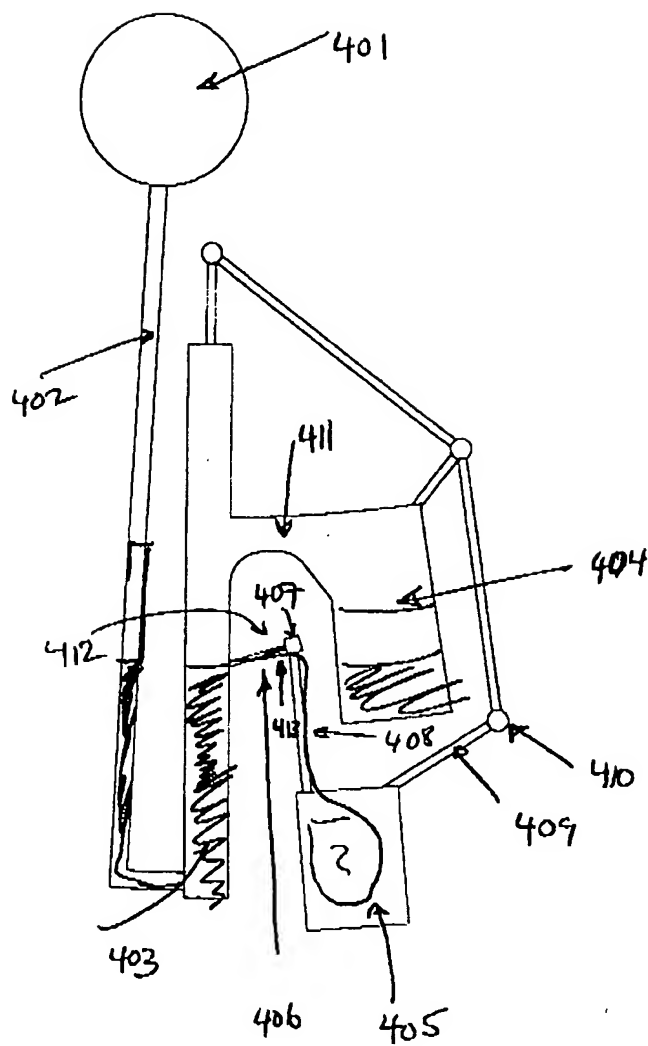


FIG. 9H

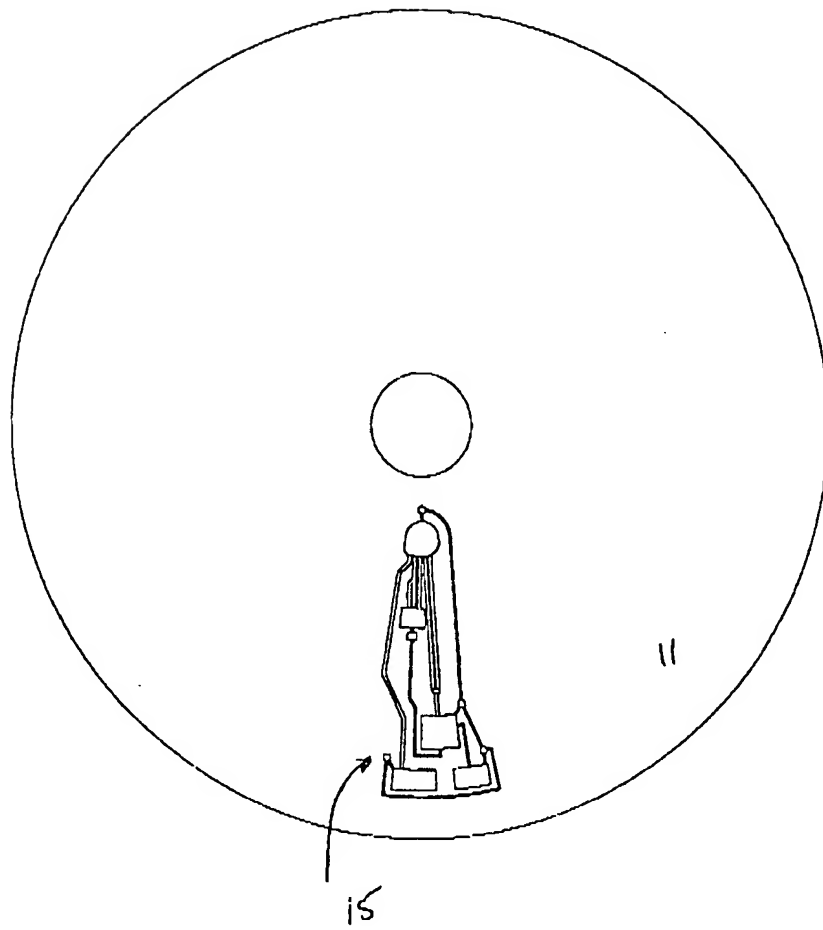


Fig. 10

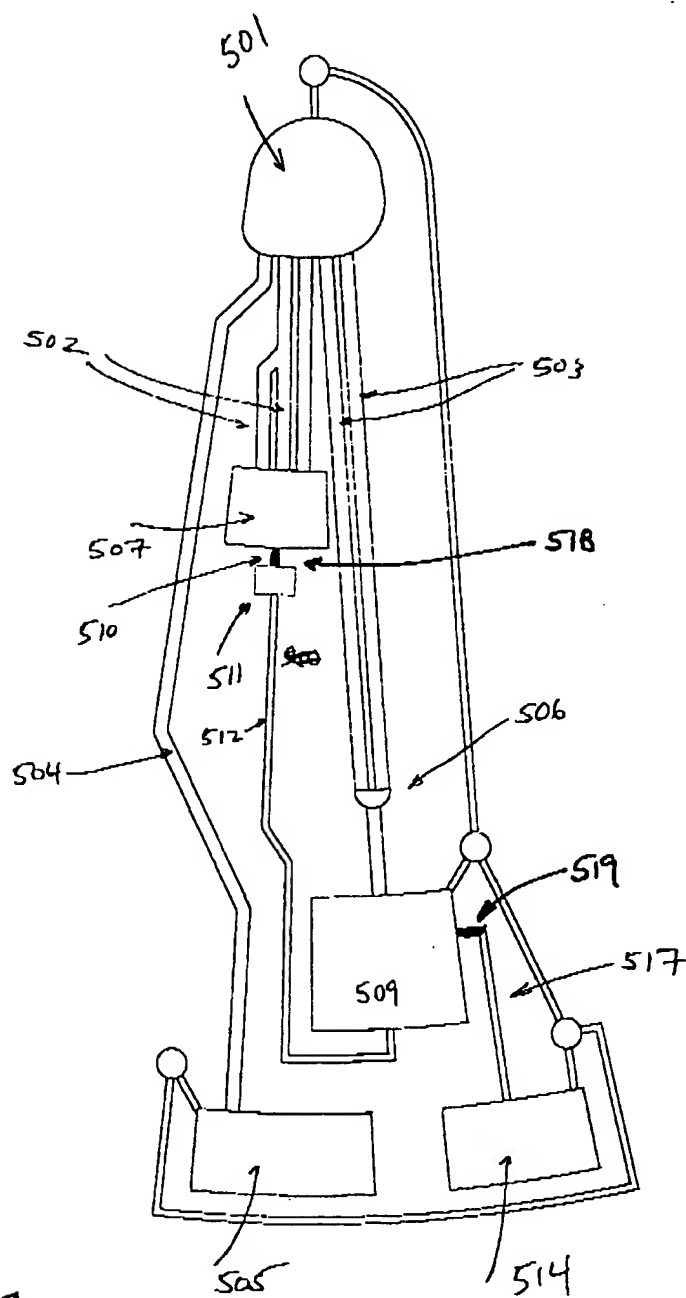


Fig. 11

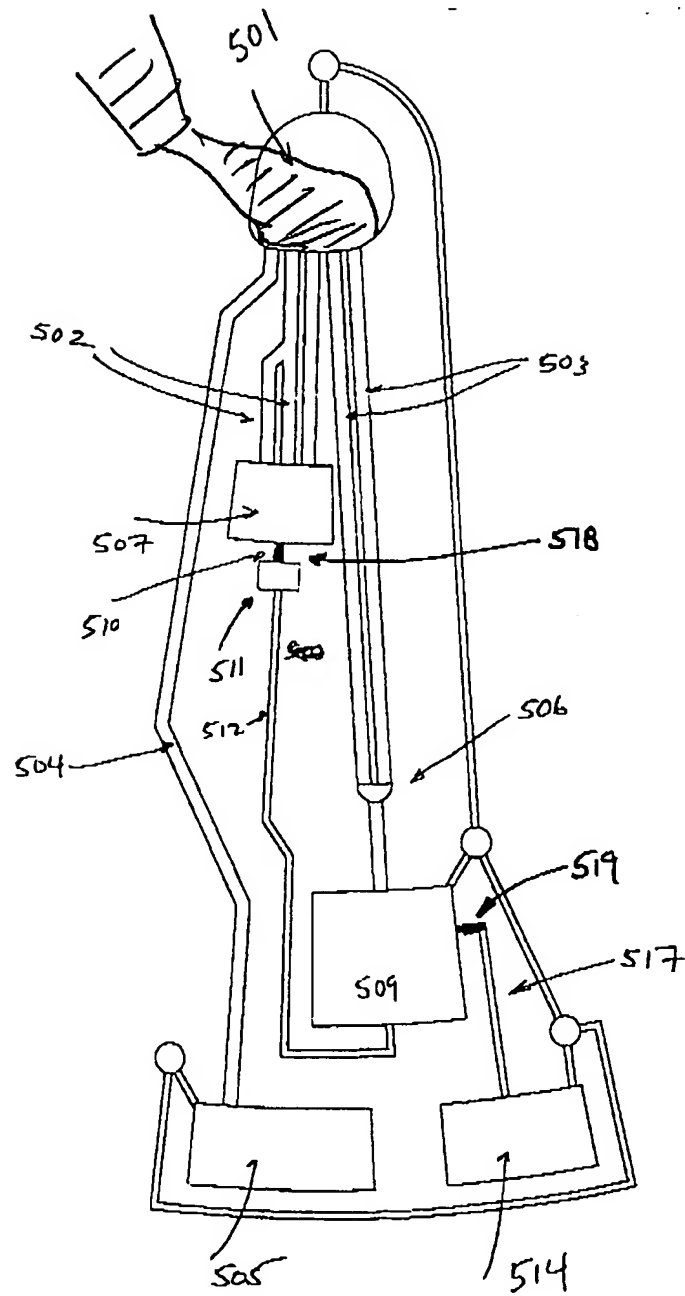
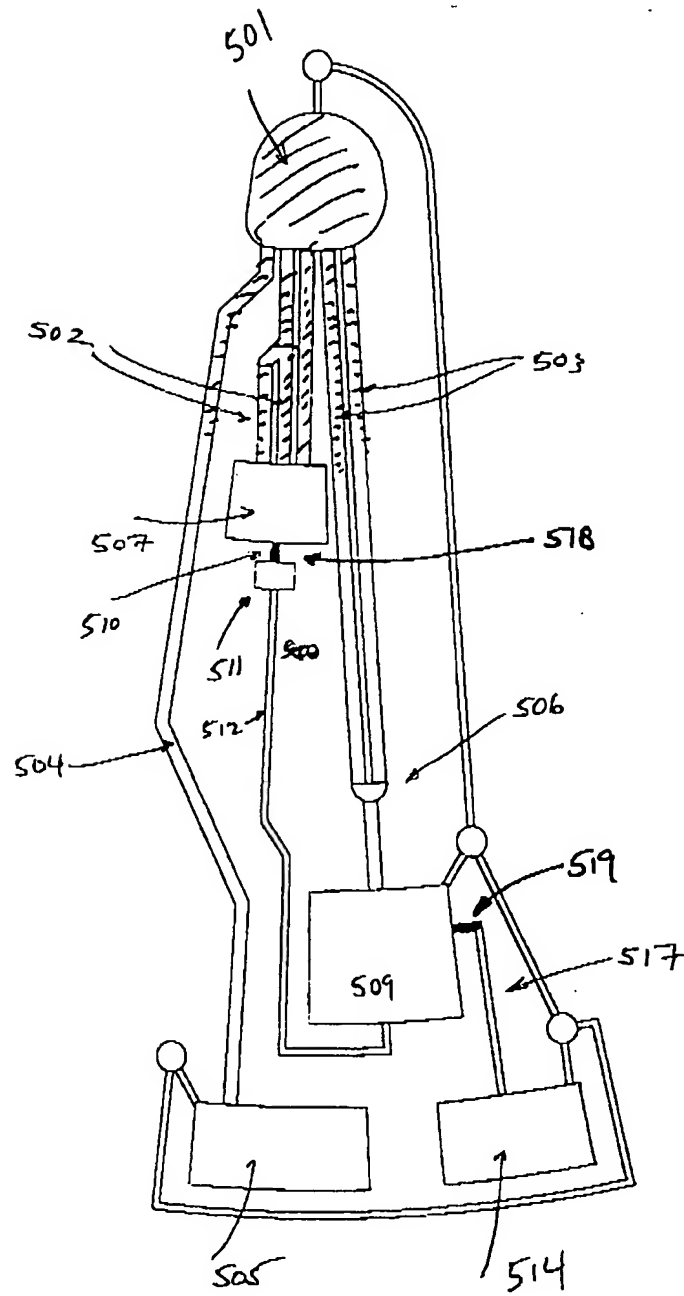


FIG. 12A



= 16. 12B

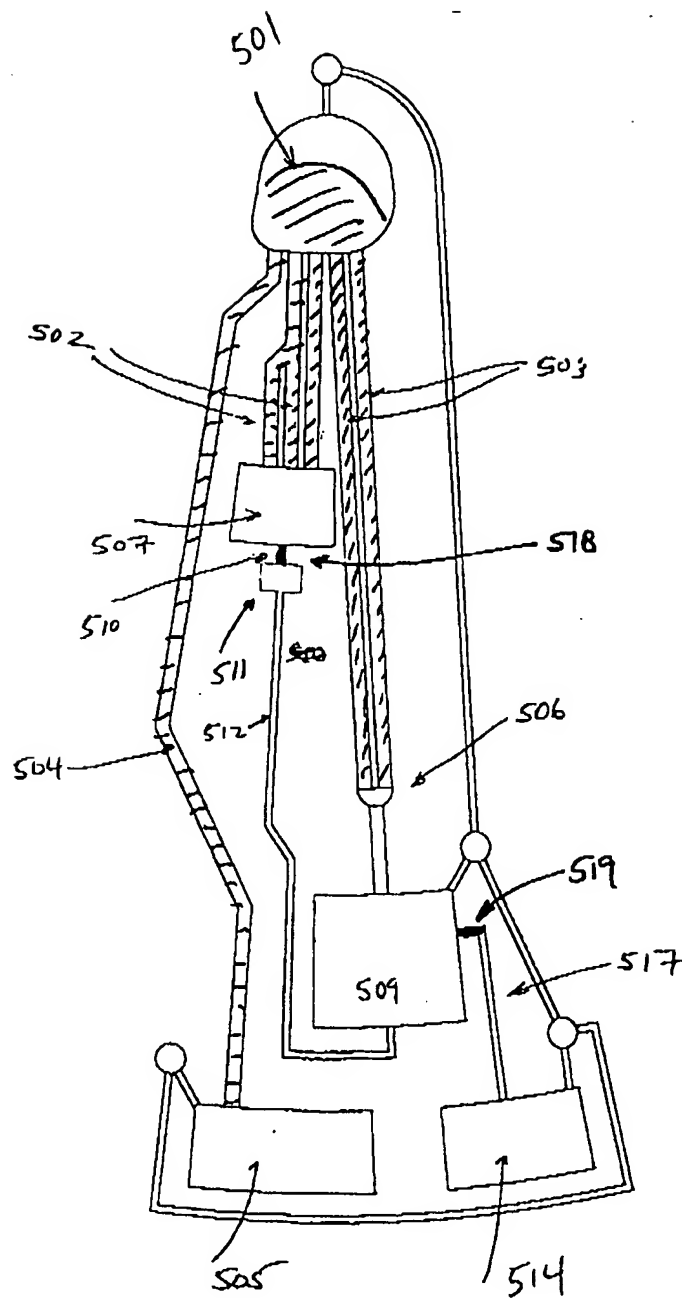


FIG. 12C



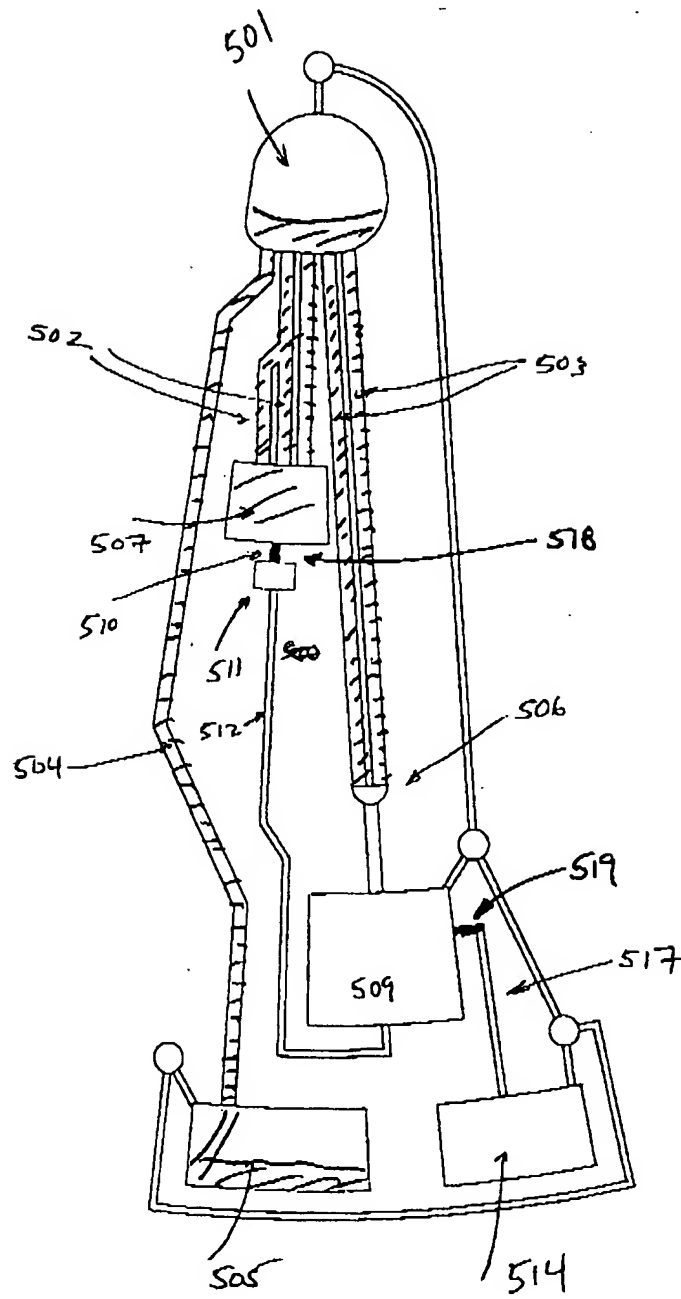


FIG. 12D

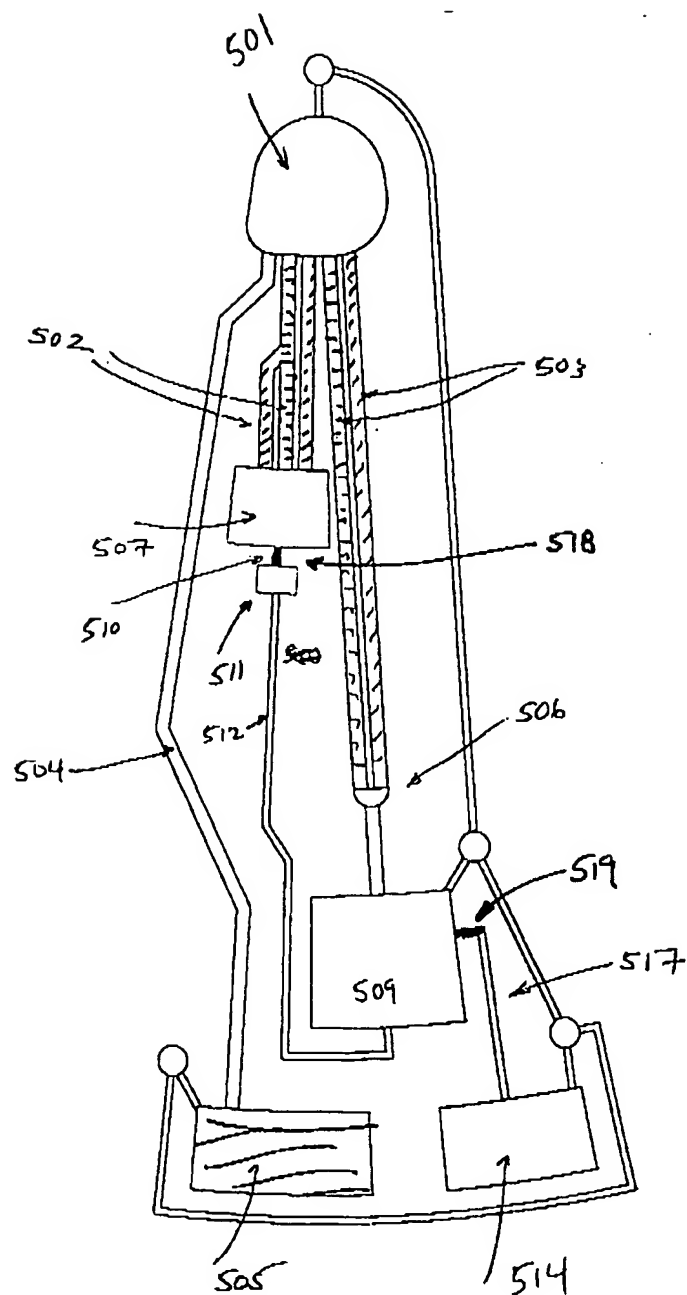
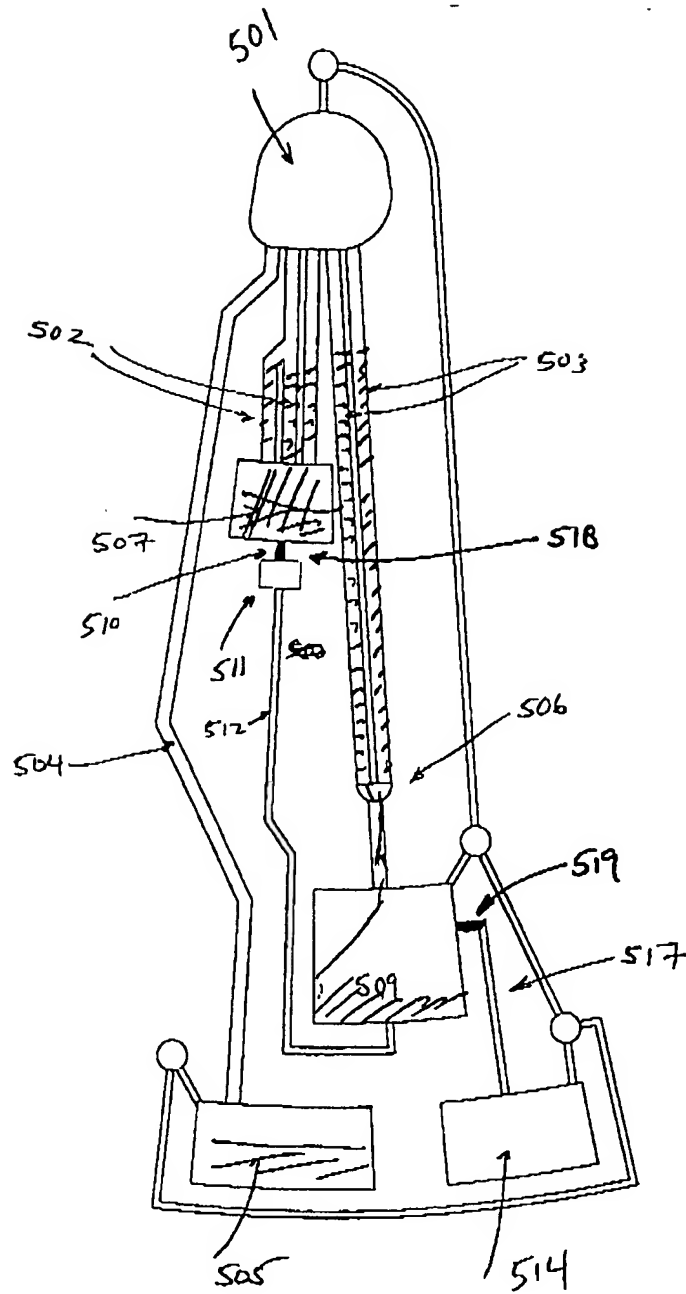


FIG. 12E



= 16. 12 F

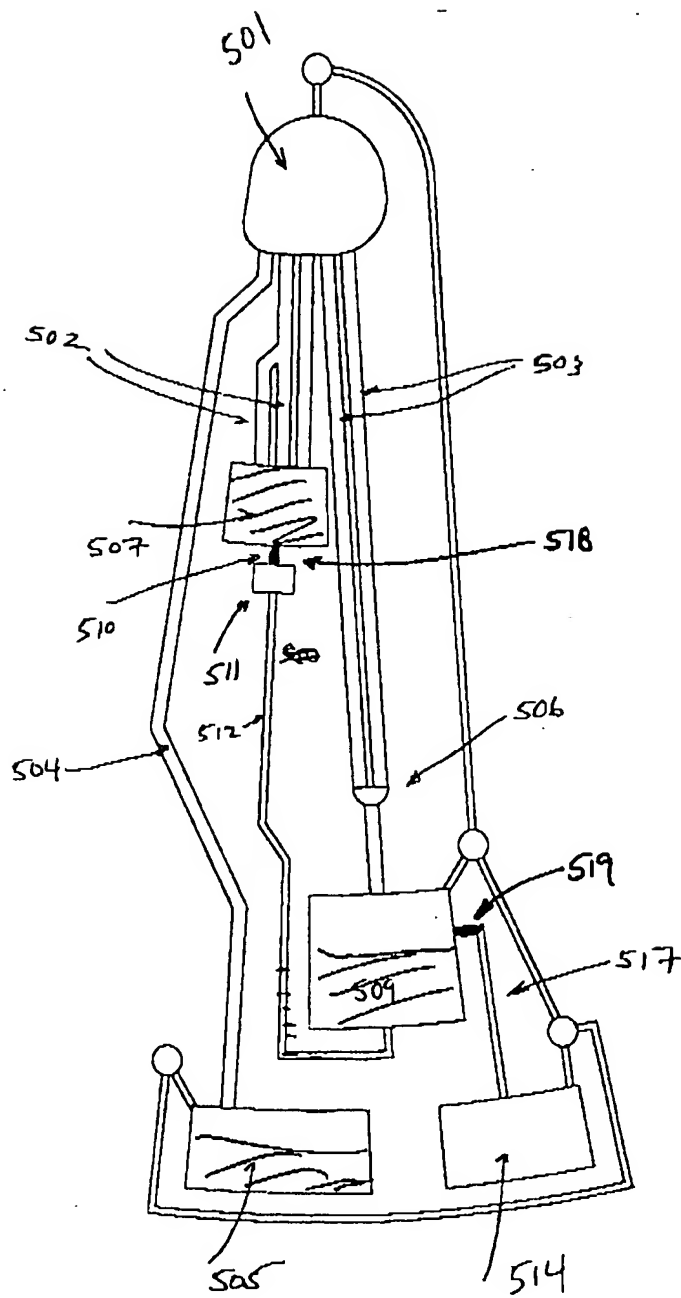
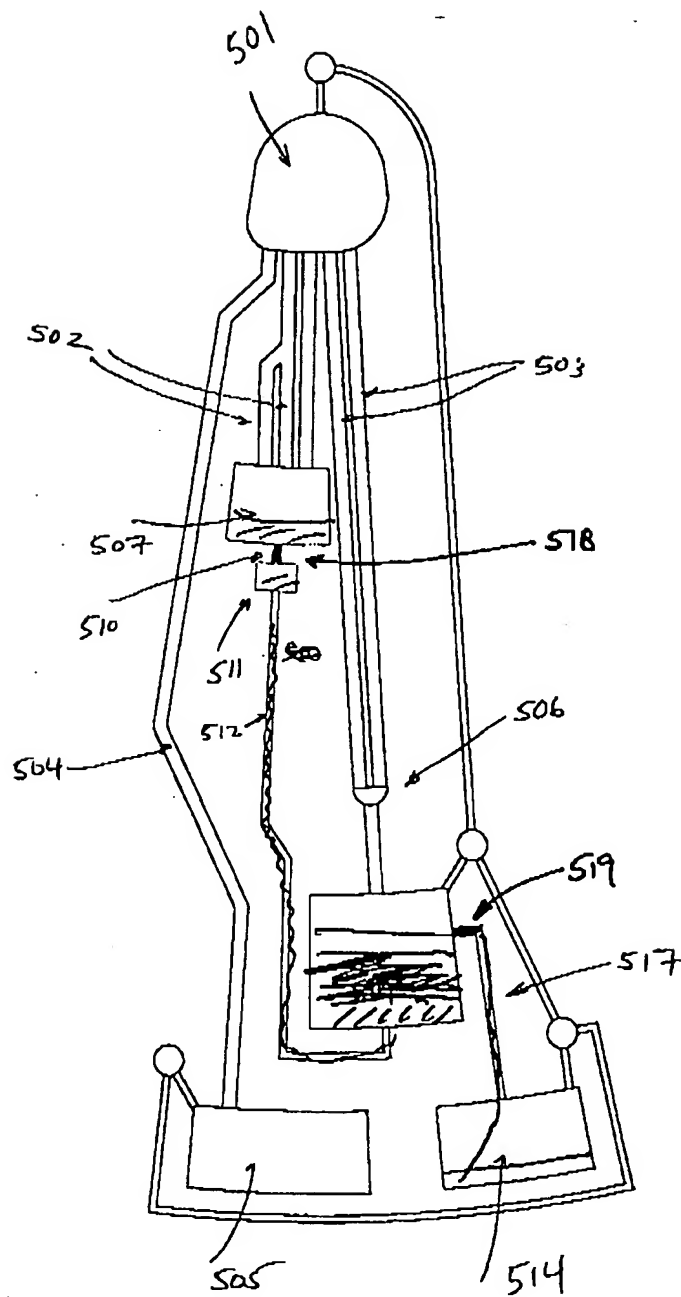


FIG. 12G





= 16. 12 I

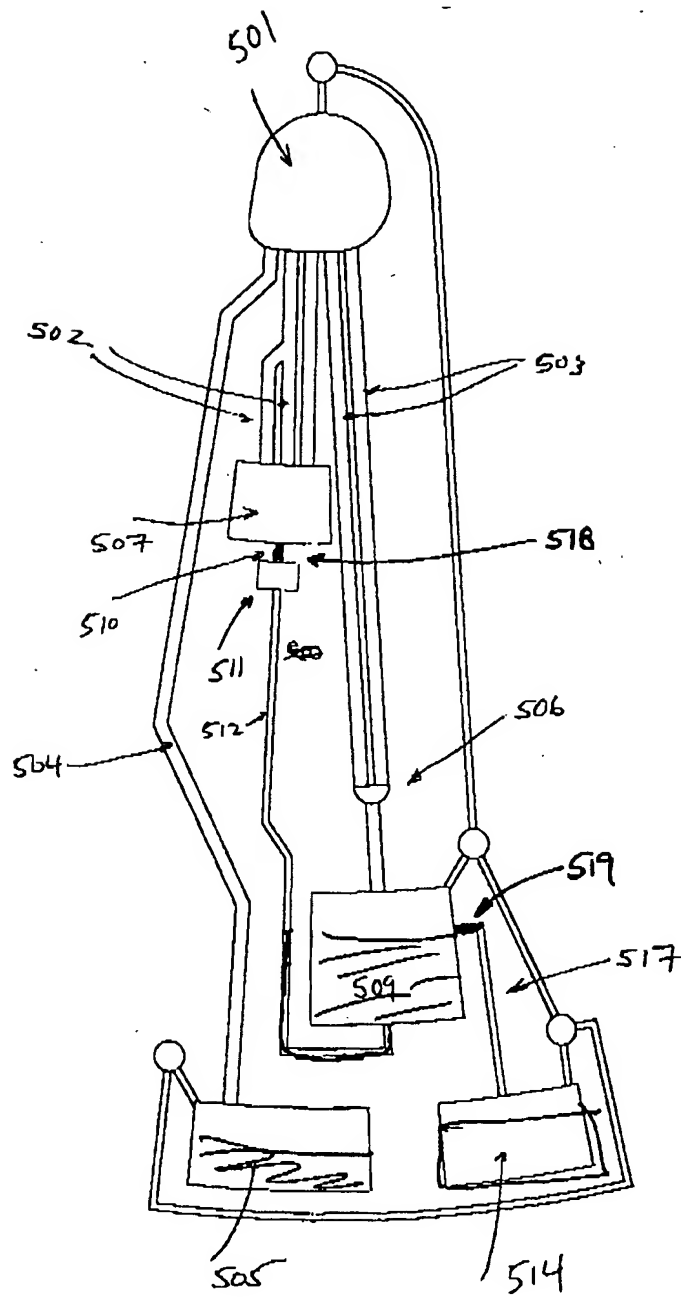


FIG. 12

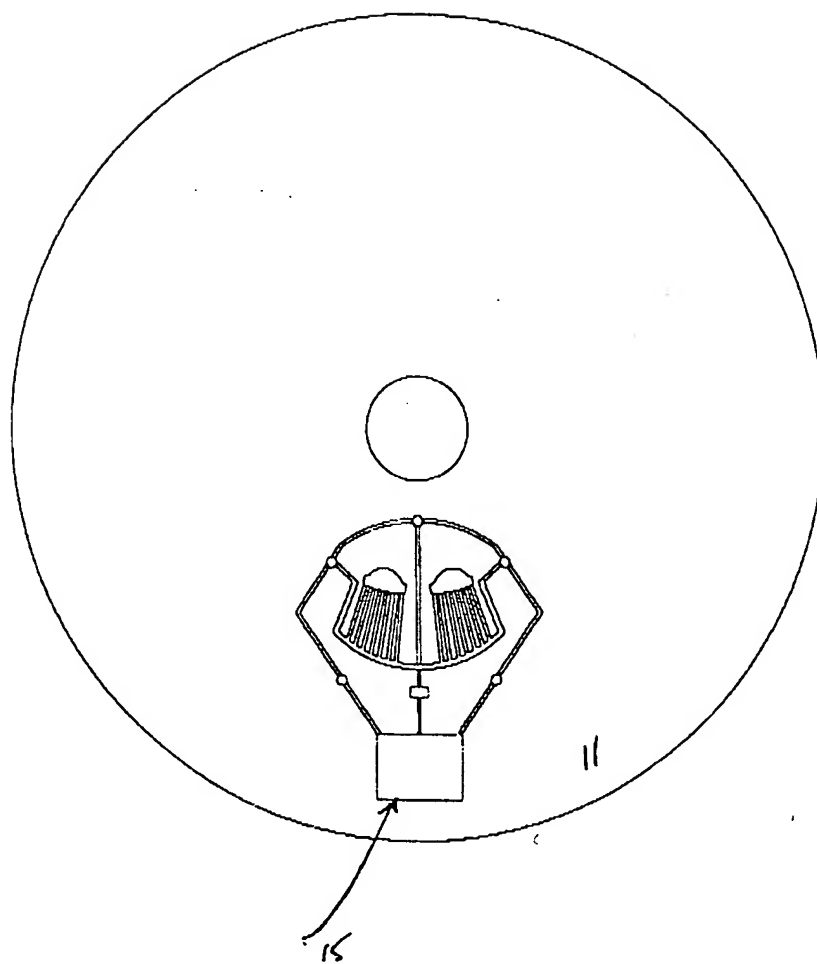


Fig. 13



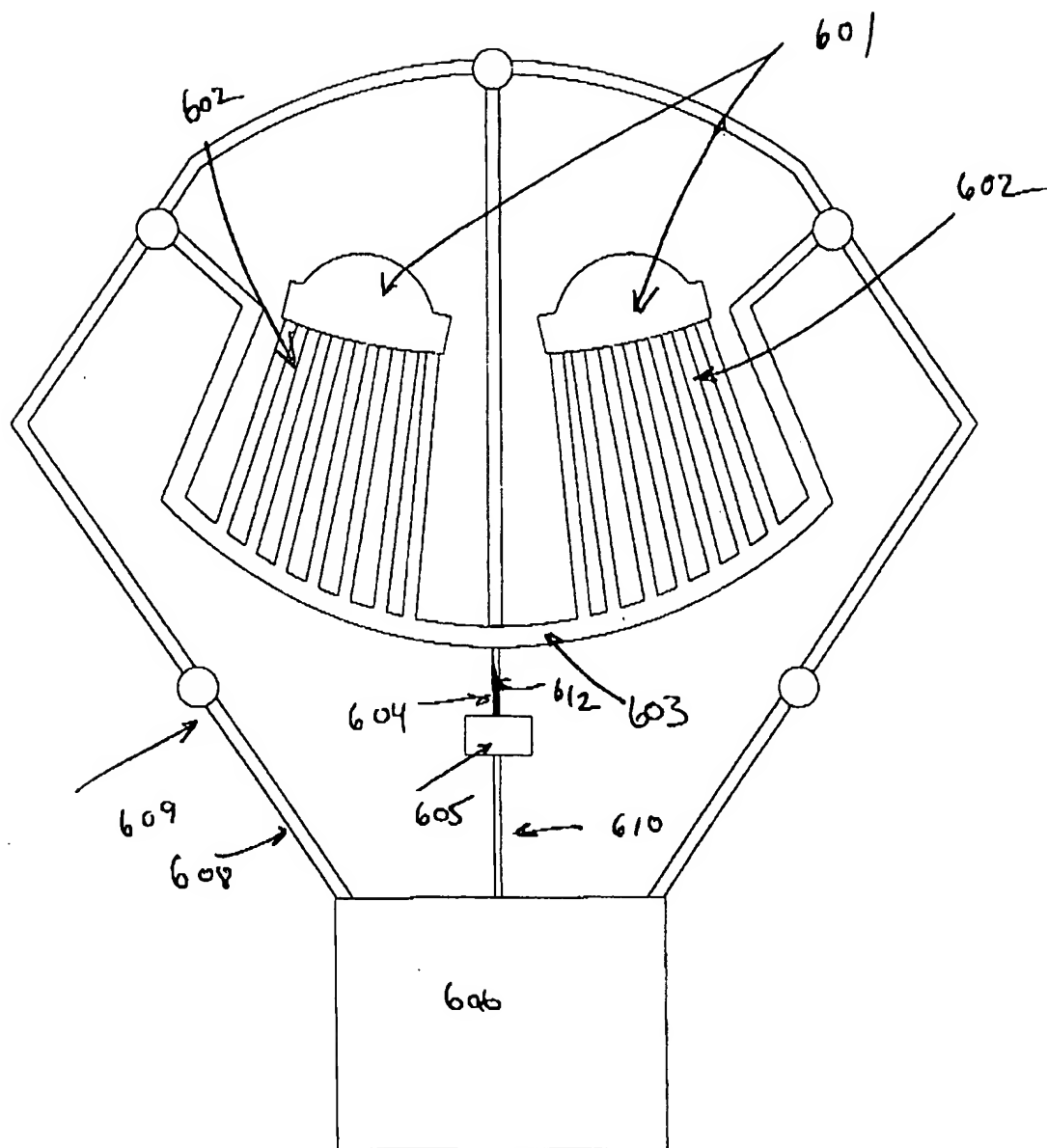


Fig. 14

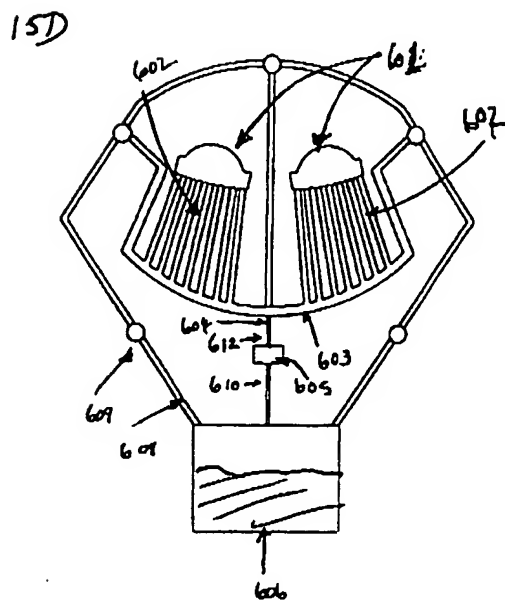
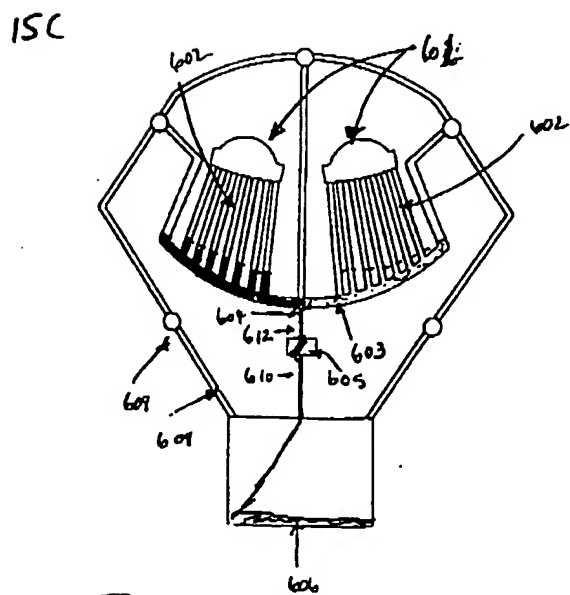
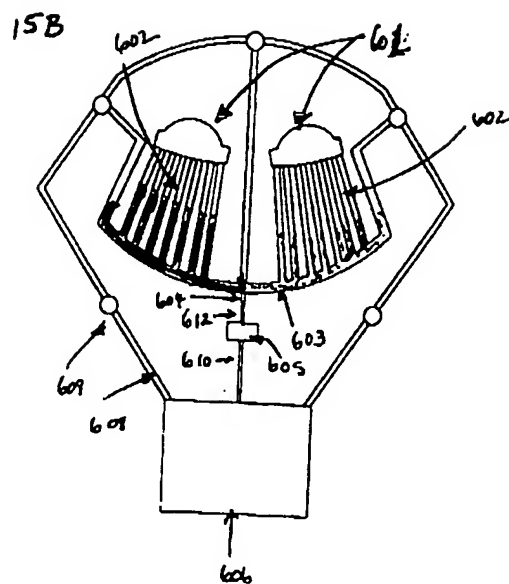
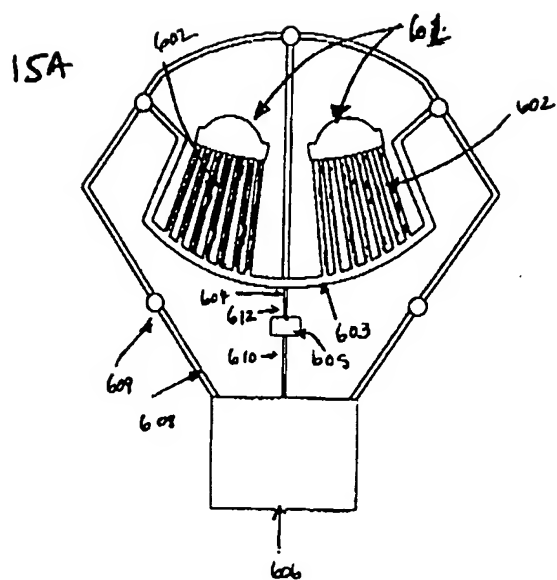


FIG. 15

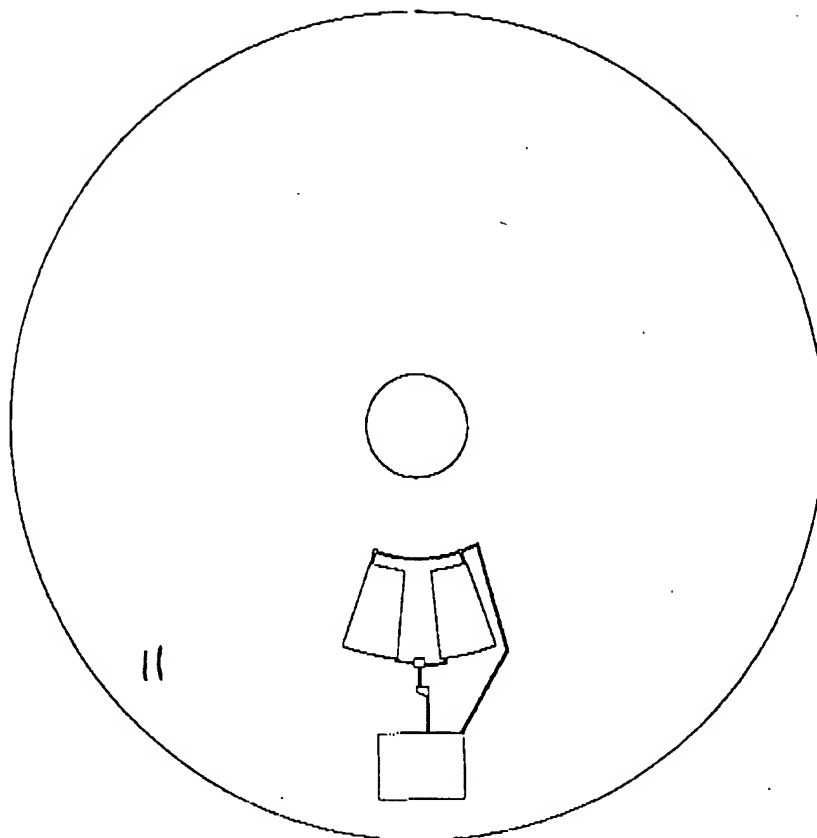


Fig. 16

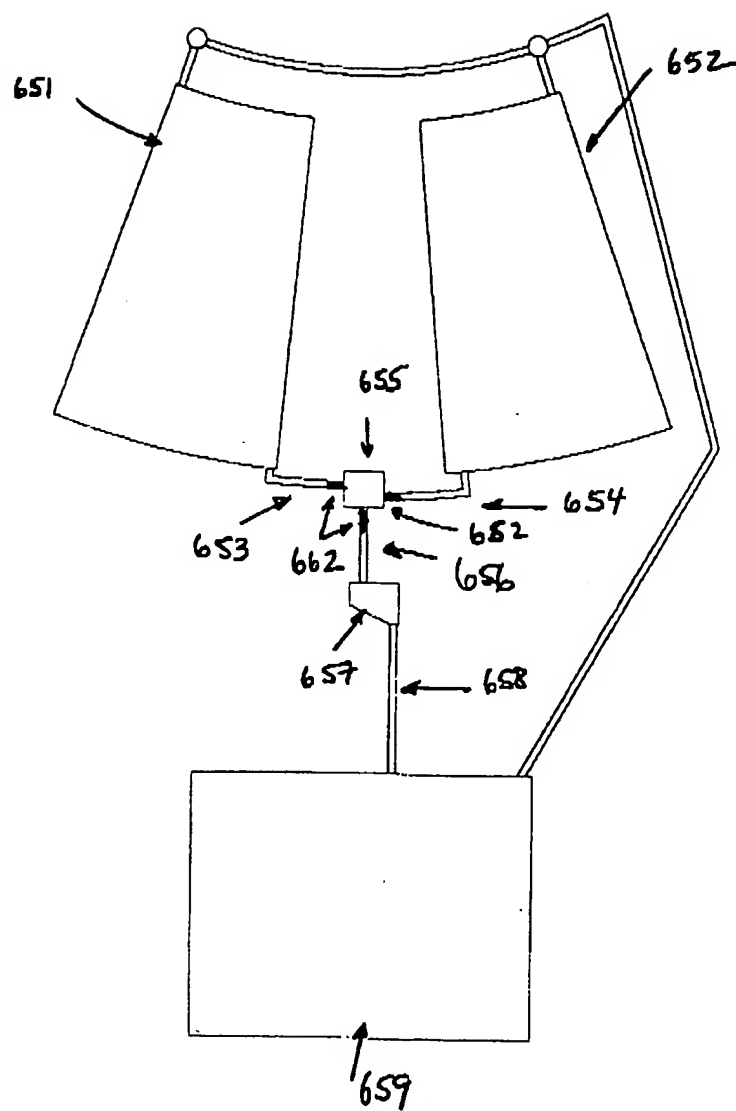
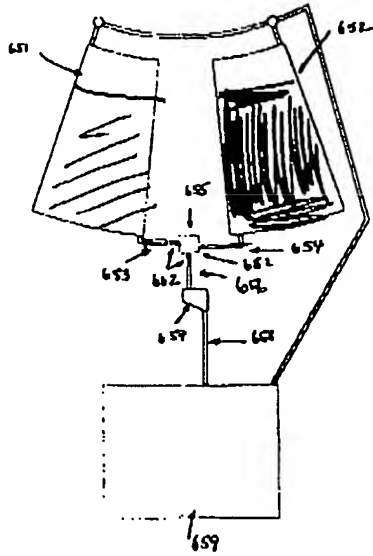
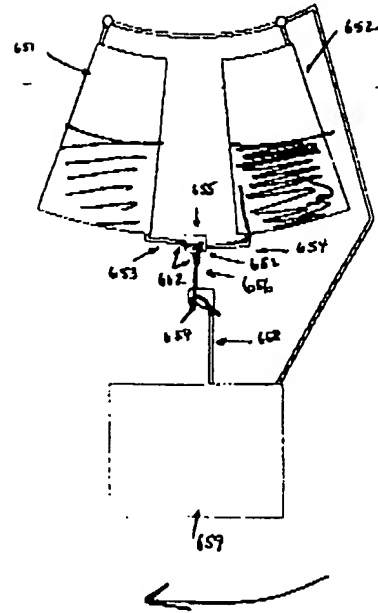


Fig. 17

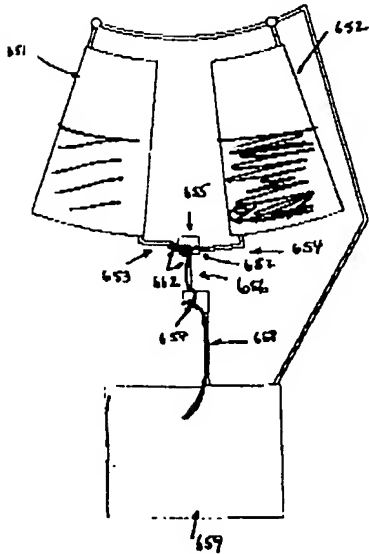
18A



18B



18C



18D

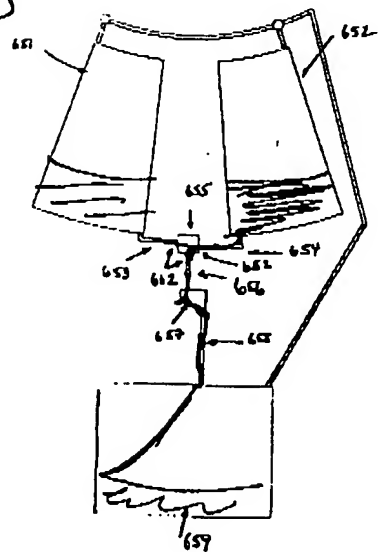


FIG. 18

18E

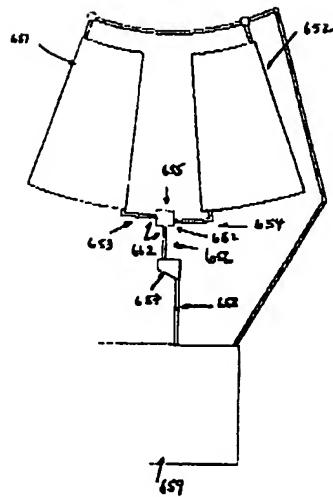
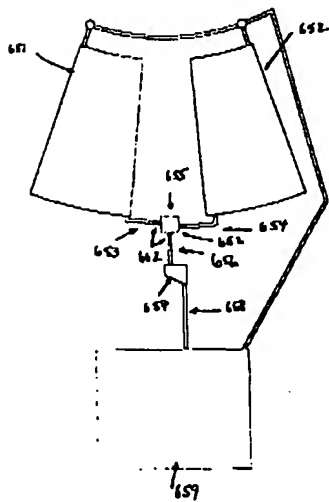
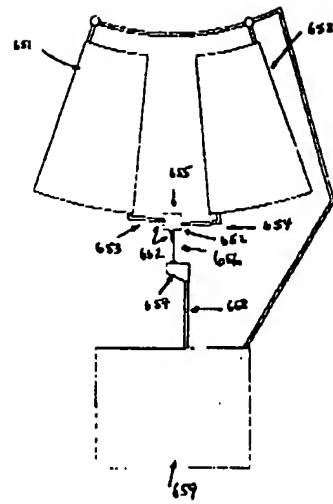
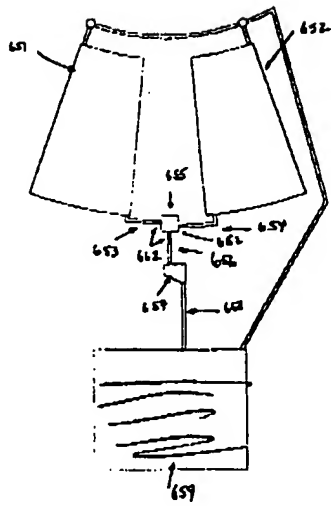


FIG. 18

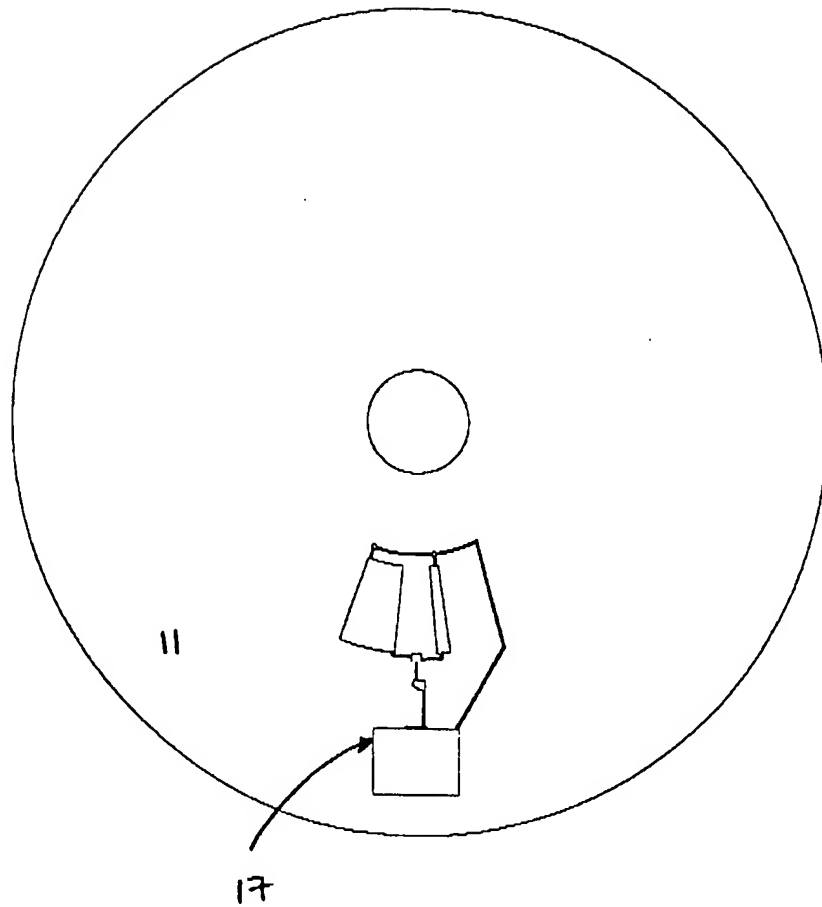


Fig. 19

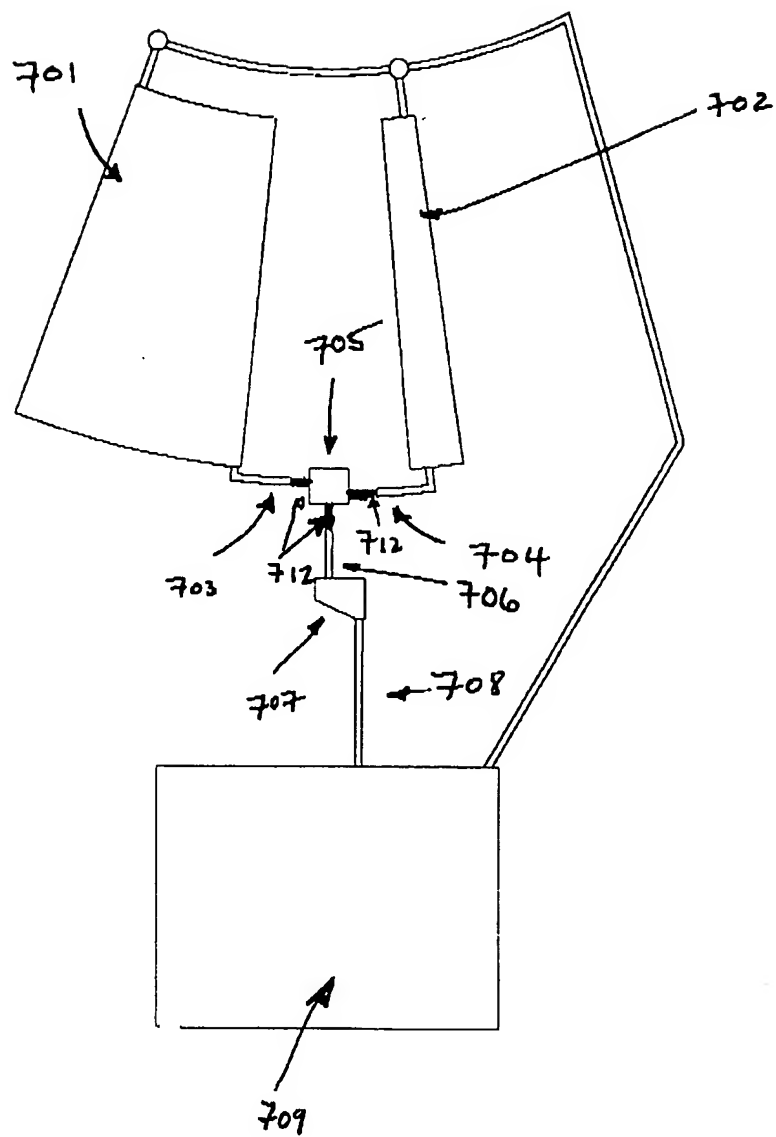


Fig. 20



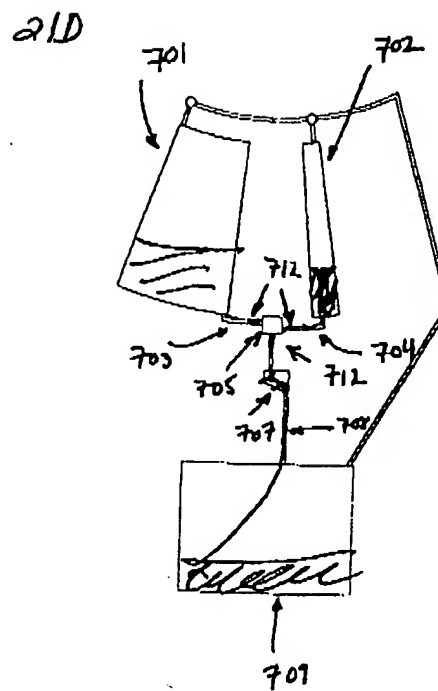
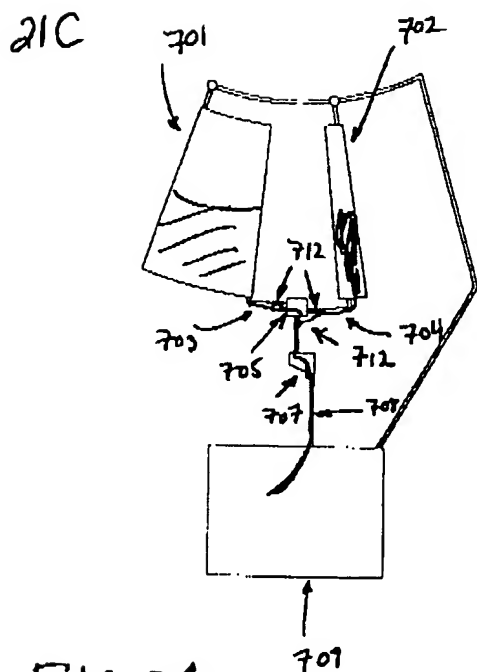
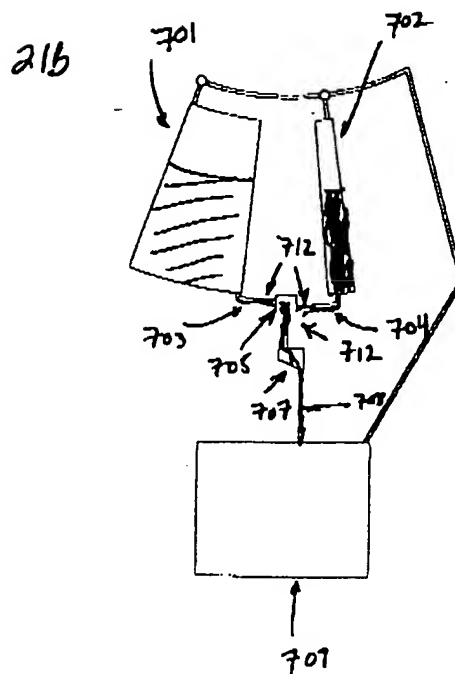
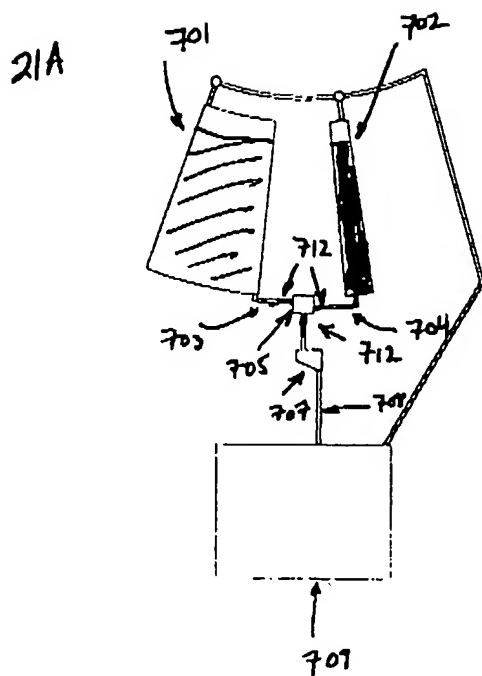


FIG. 29

21E

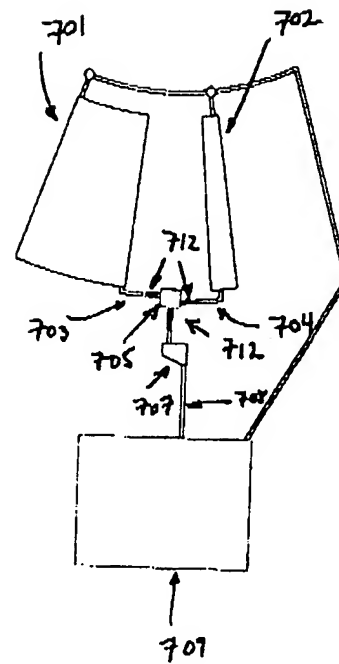
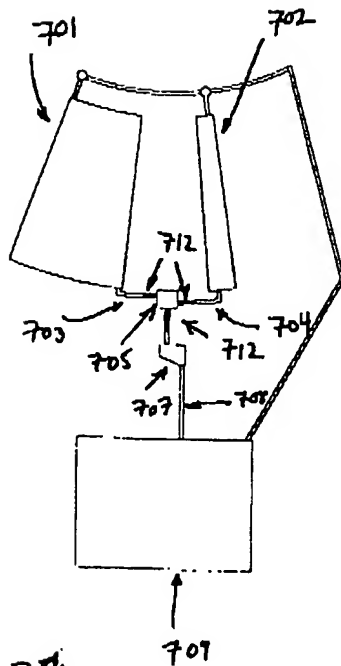
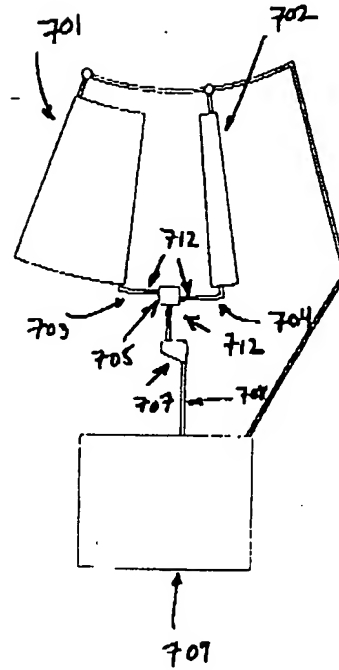
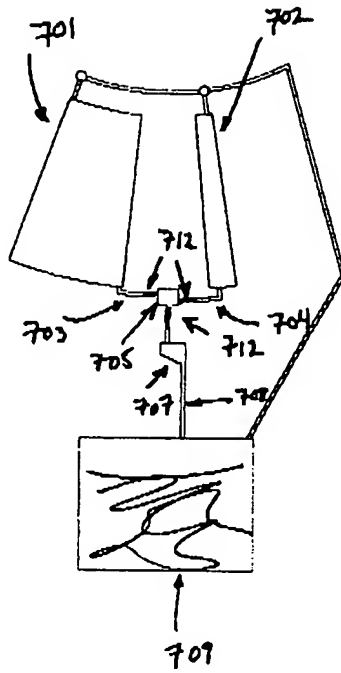


FIG. 24

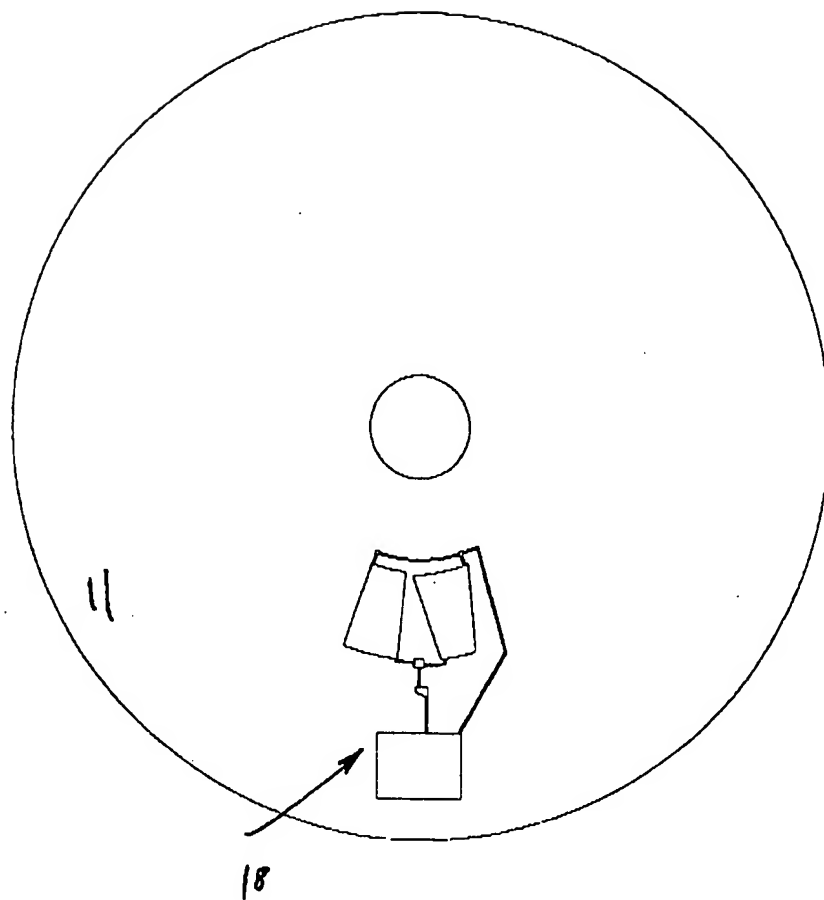


Fig 22

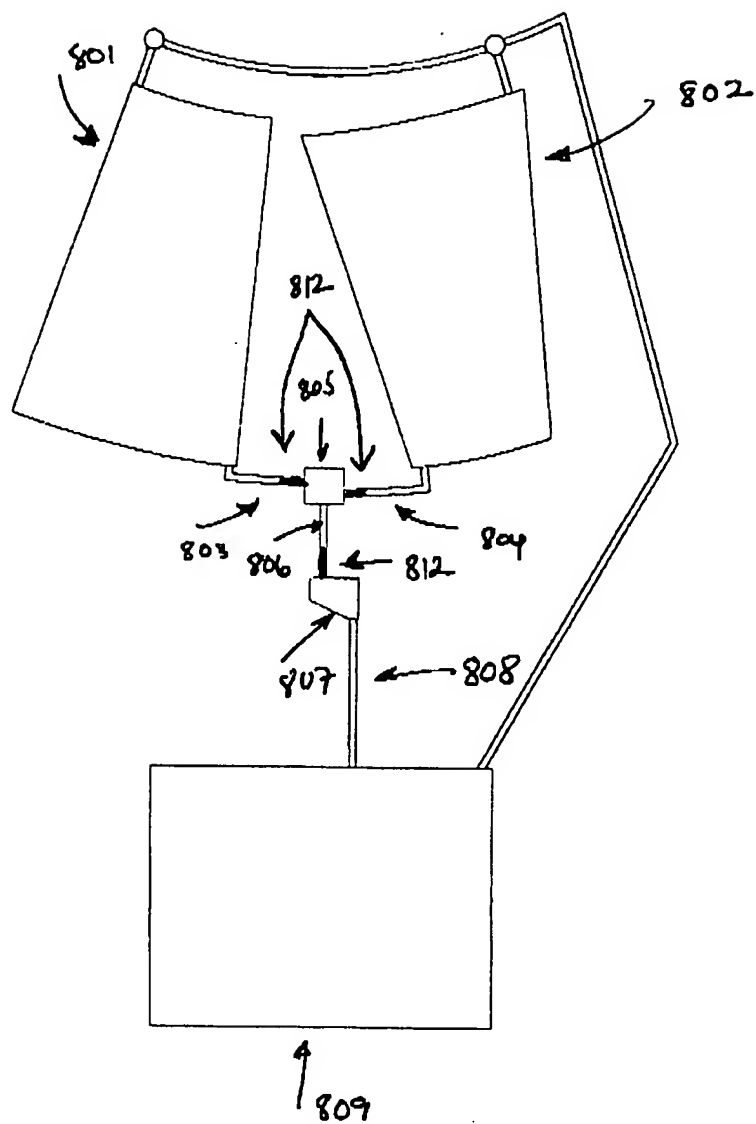
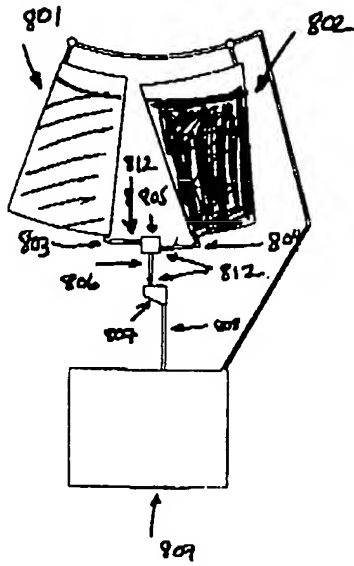
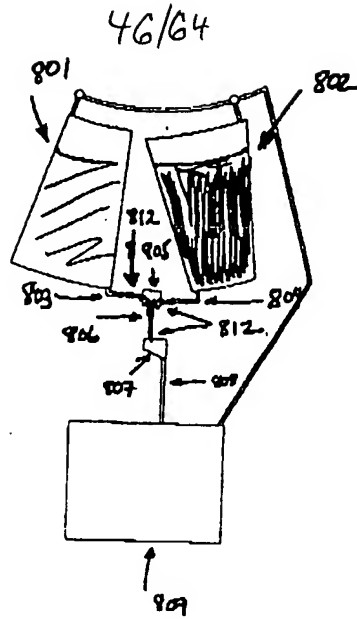


Fig 23

24A



24B



24C

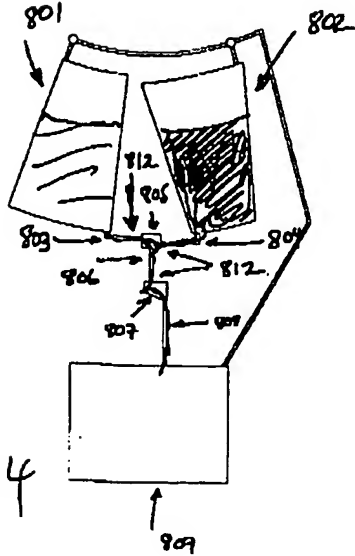
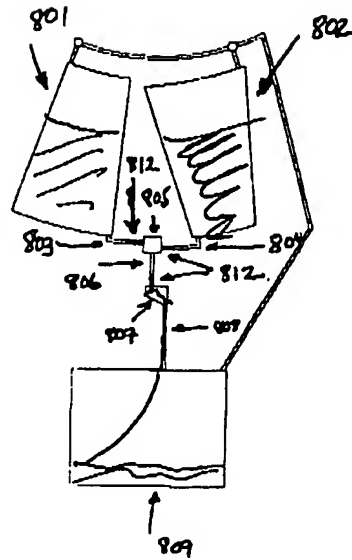
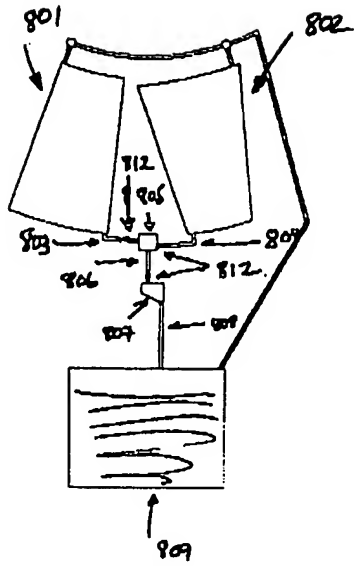


FIG. 24

24D



24E



47/64

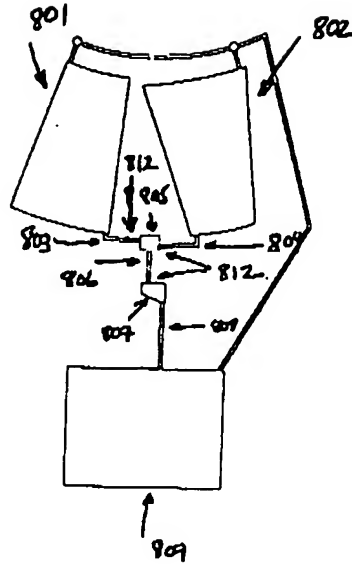
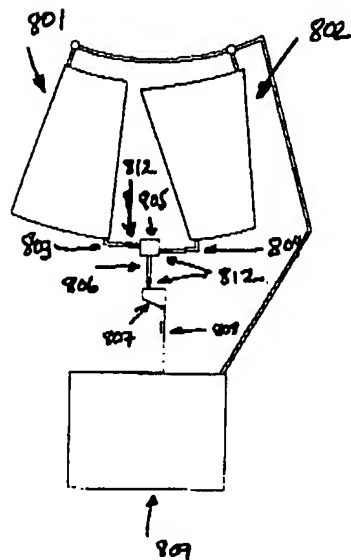
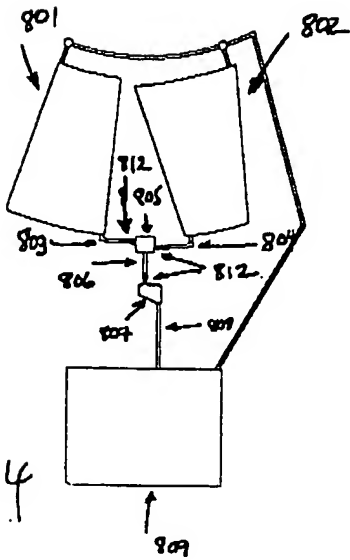


FIG. 24



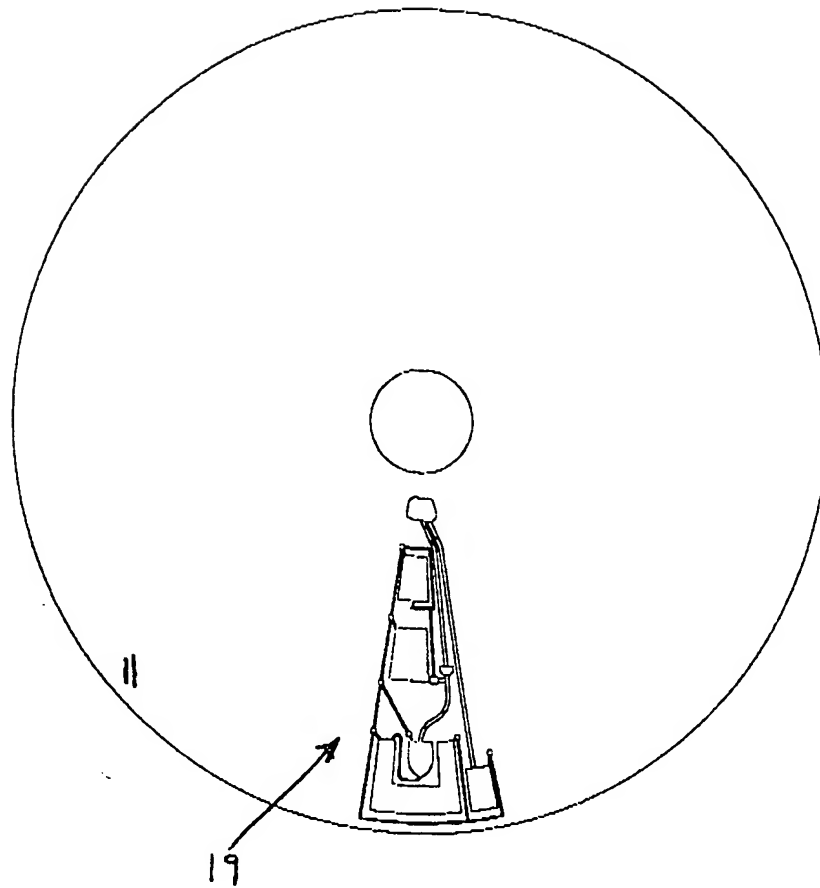


FIG. 25

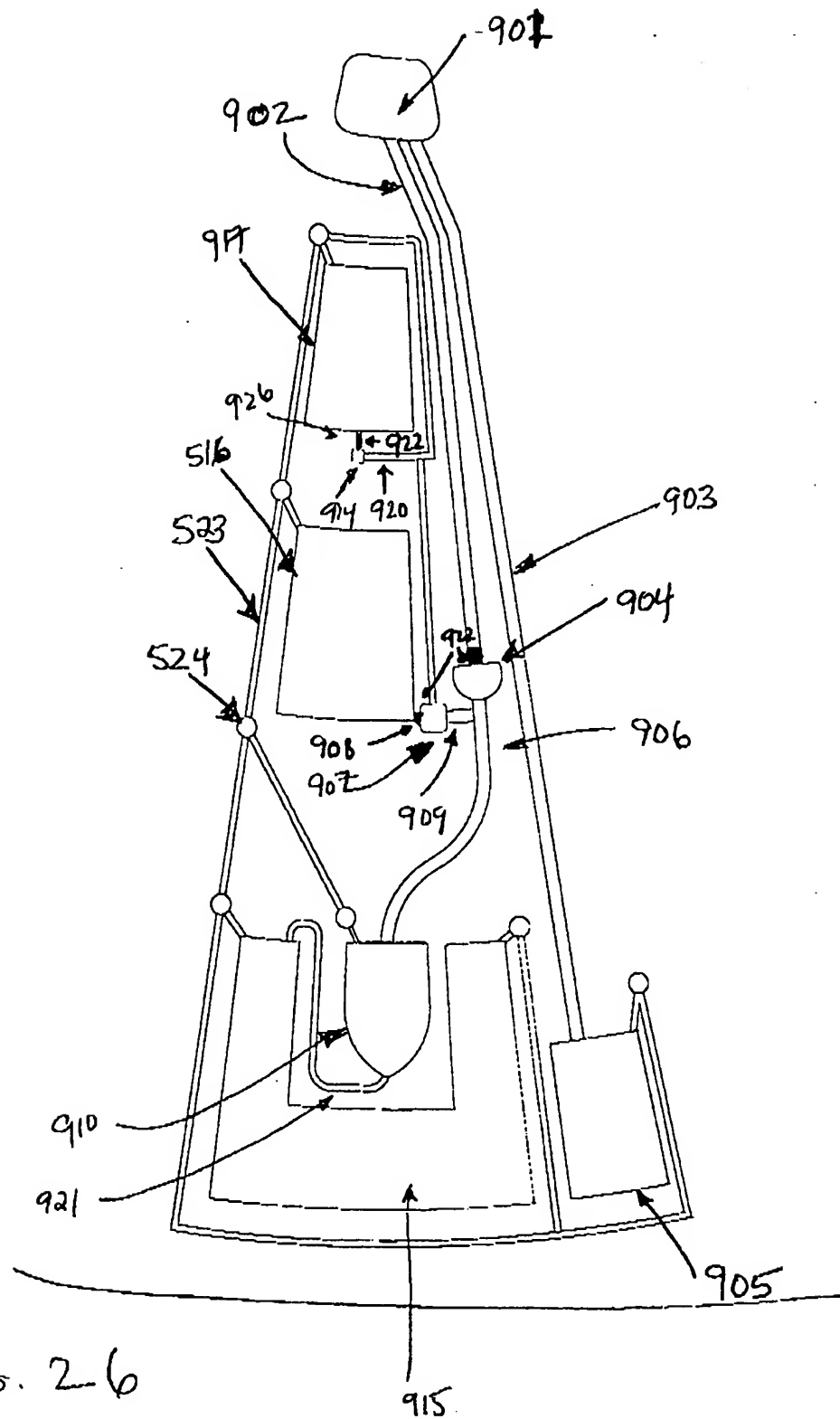


FIG. 26



27A

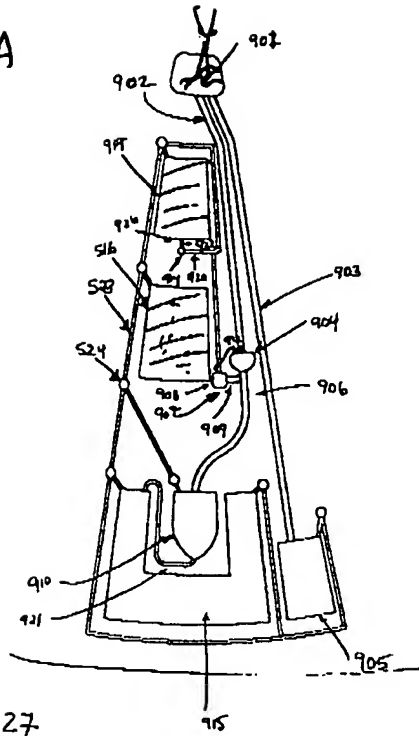


FIG. 27

27B

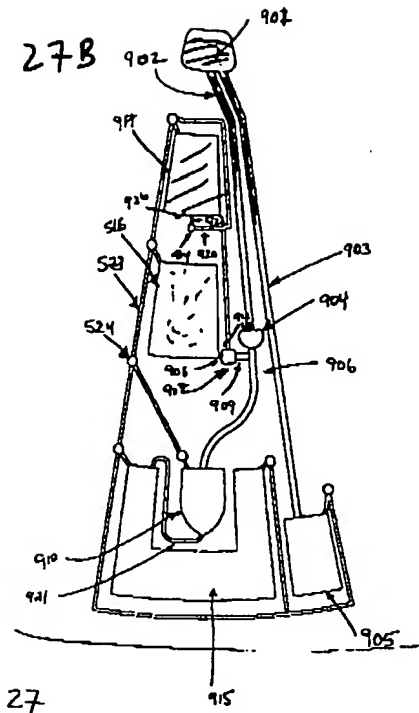


FIG. 27

27C

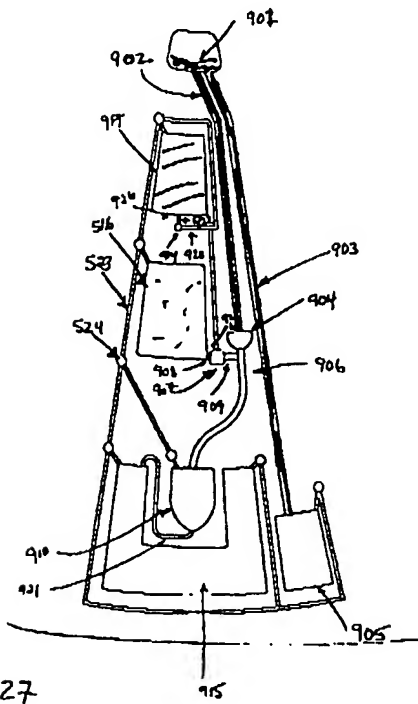


FIG. 27

27D

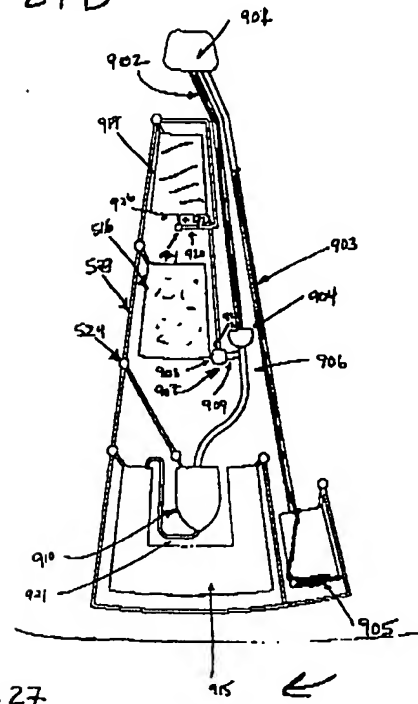
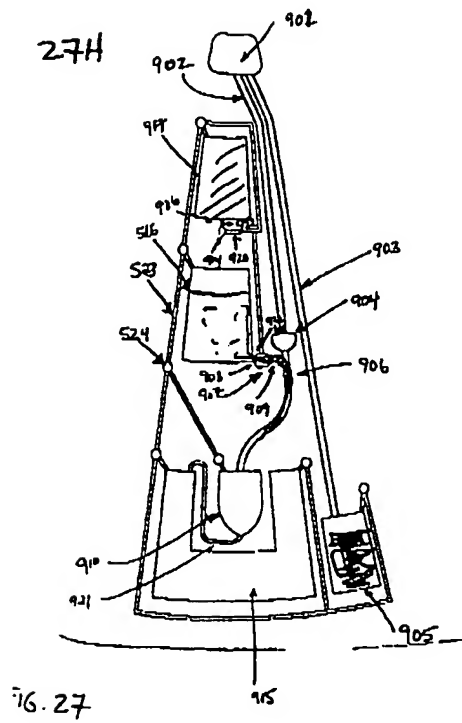
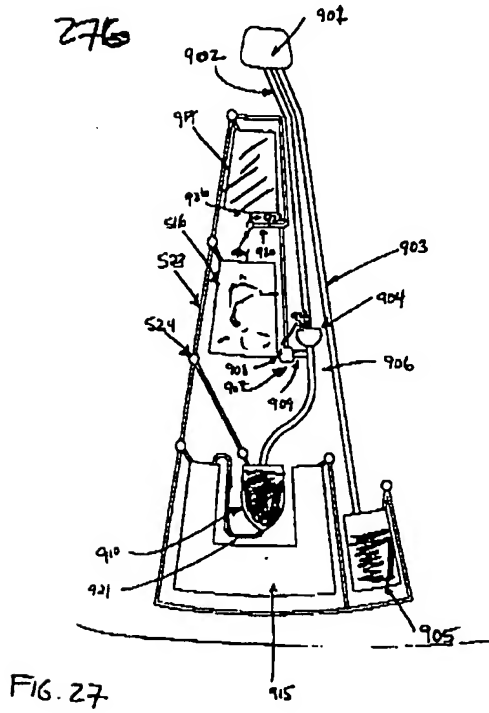
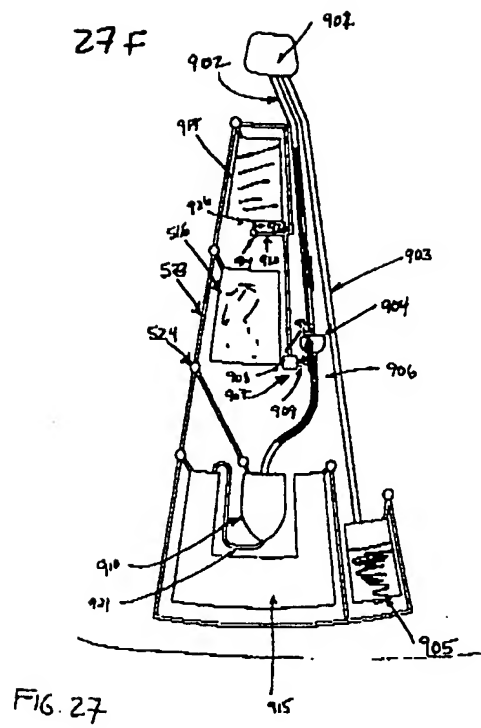
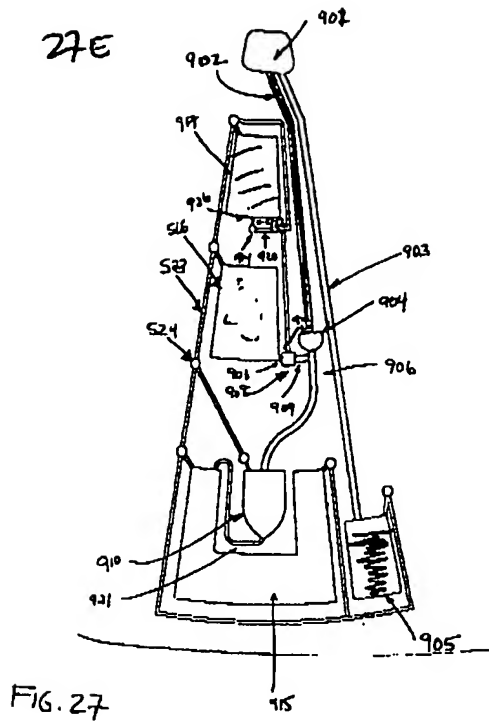
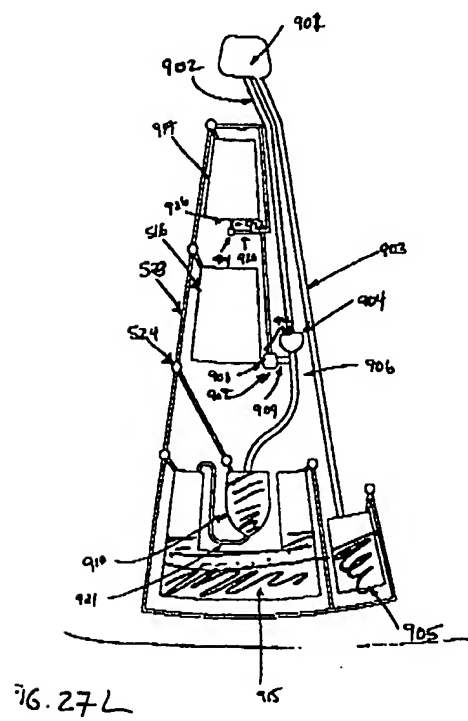
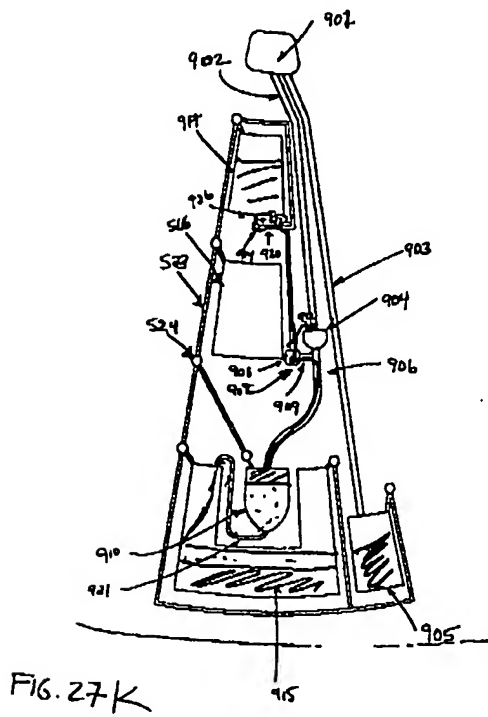
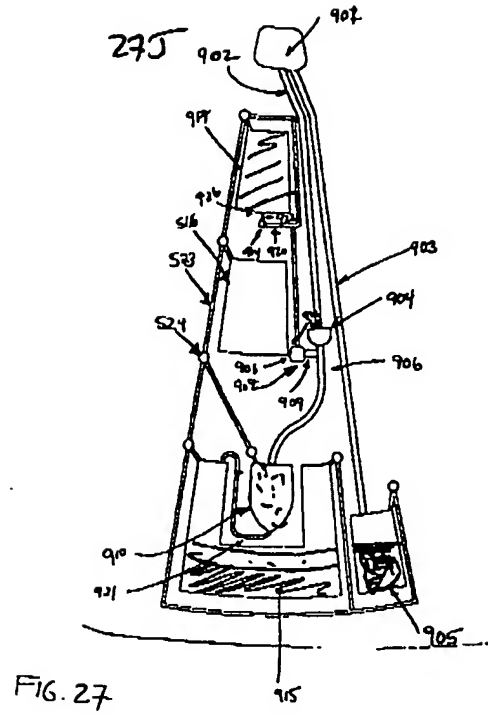
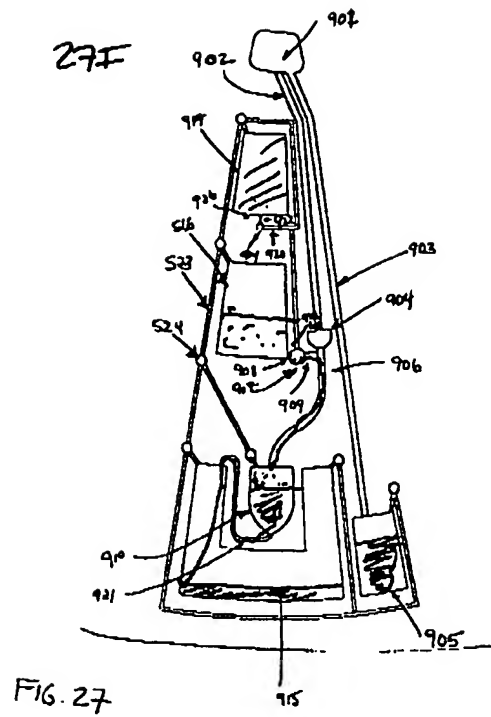


FIG. 27





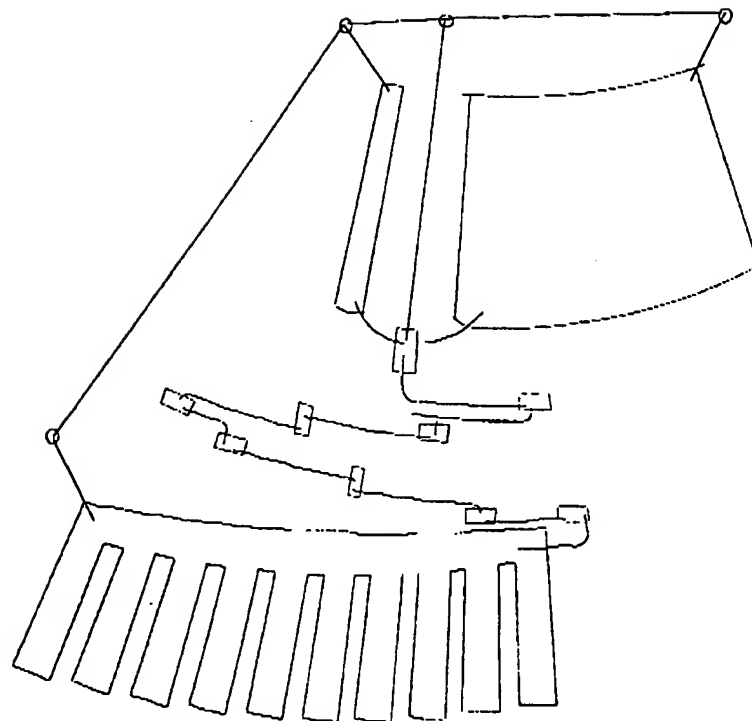
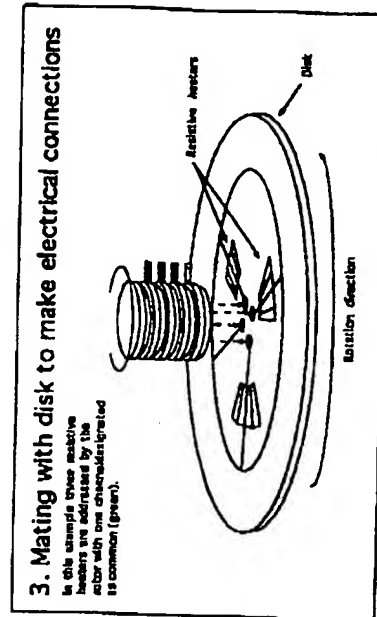
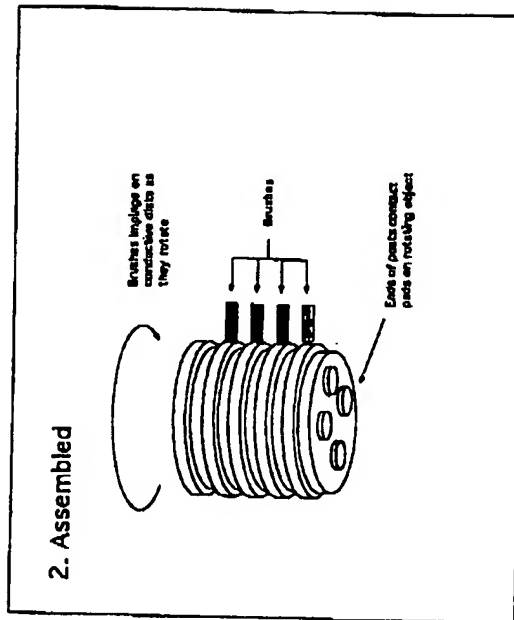
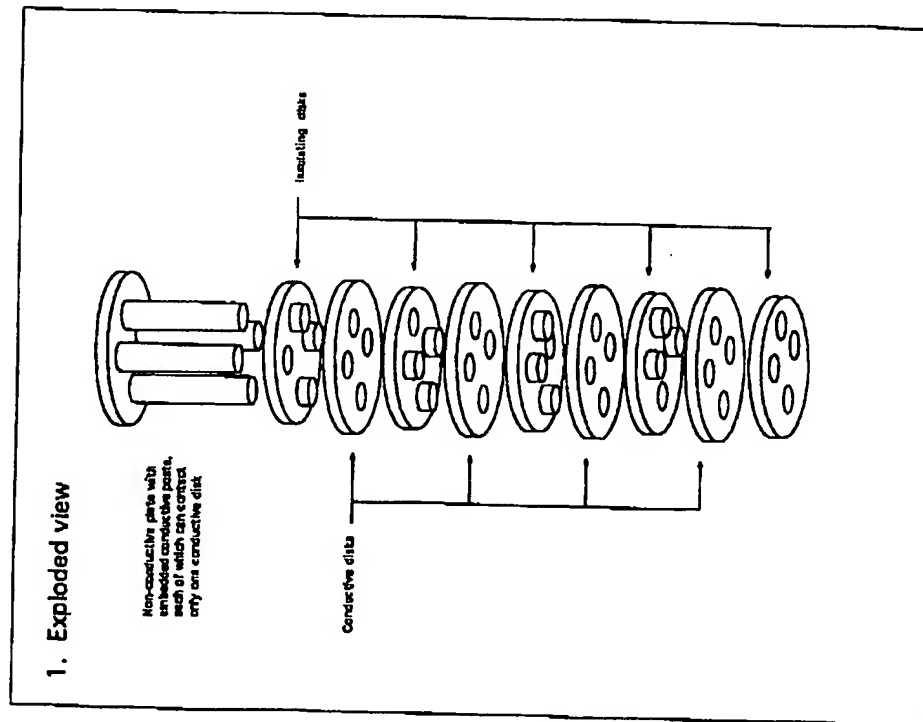
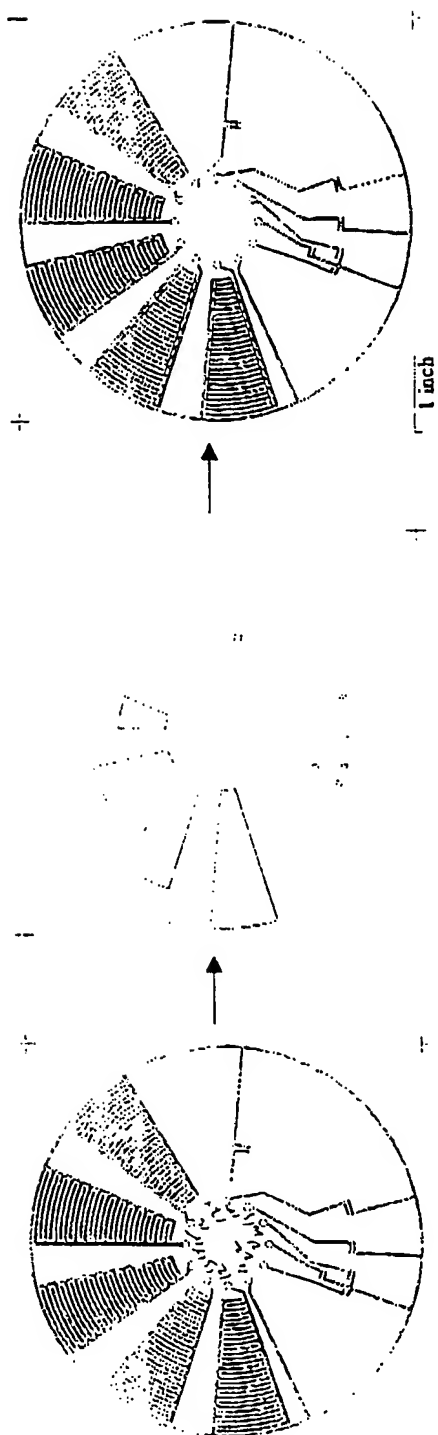


FIG. 28

Means for delivering electrical signals to a rotating object

Fig 29



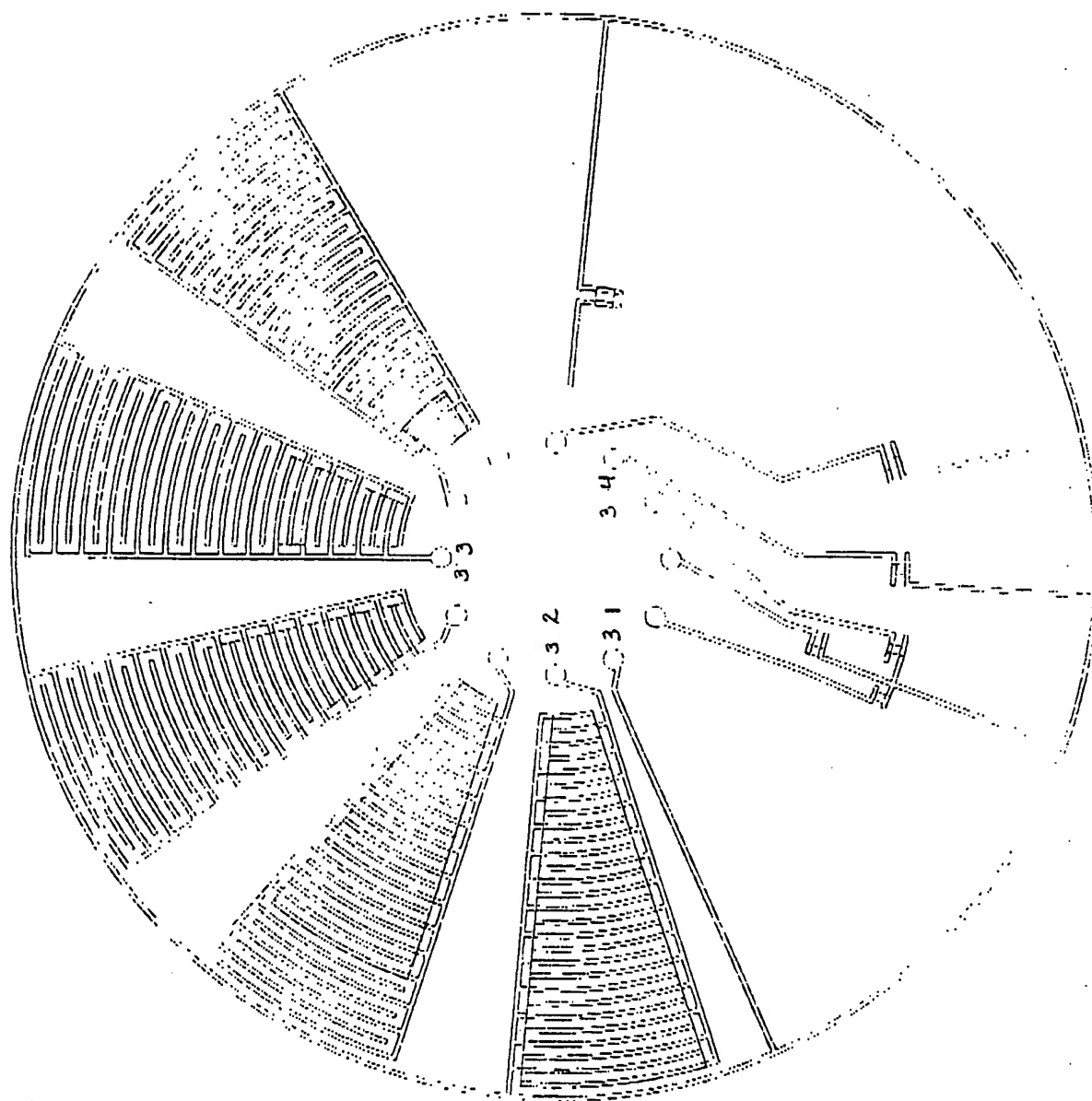


Conductive silver ink is screen-printed on heat-stabilized polyester film to form leads. The screened parts are cured at 120°C for 10 minutes.

Resistive carbon ink is screen-printed over the silver leads to form heaters. The screened parts are cured at 120°C for 10 minutes.

Final circuit on polyester film.

Fig. 30



1 inch

Fig. 31

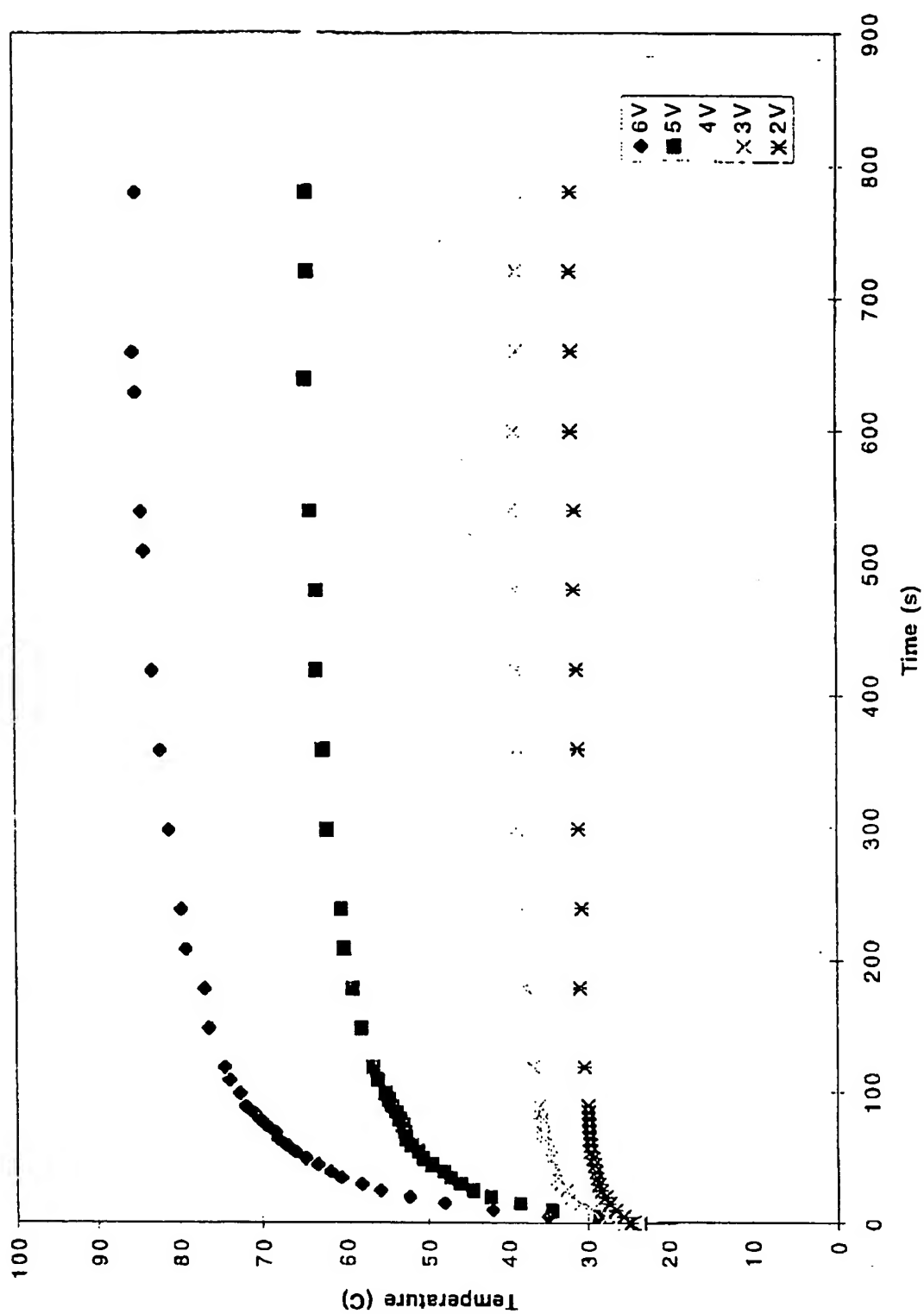


Fig. 32



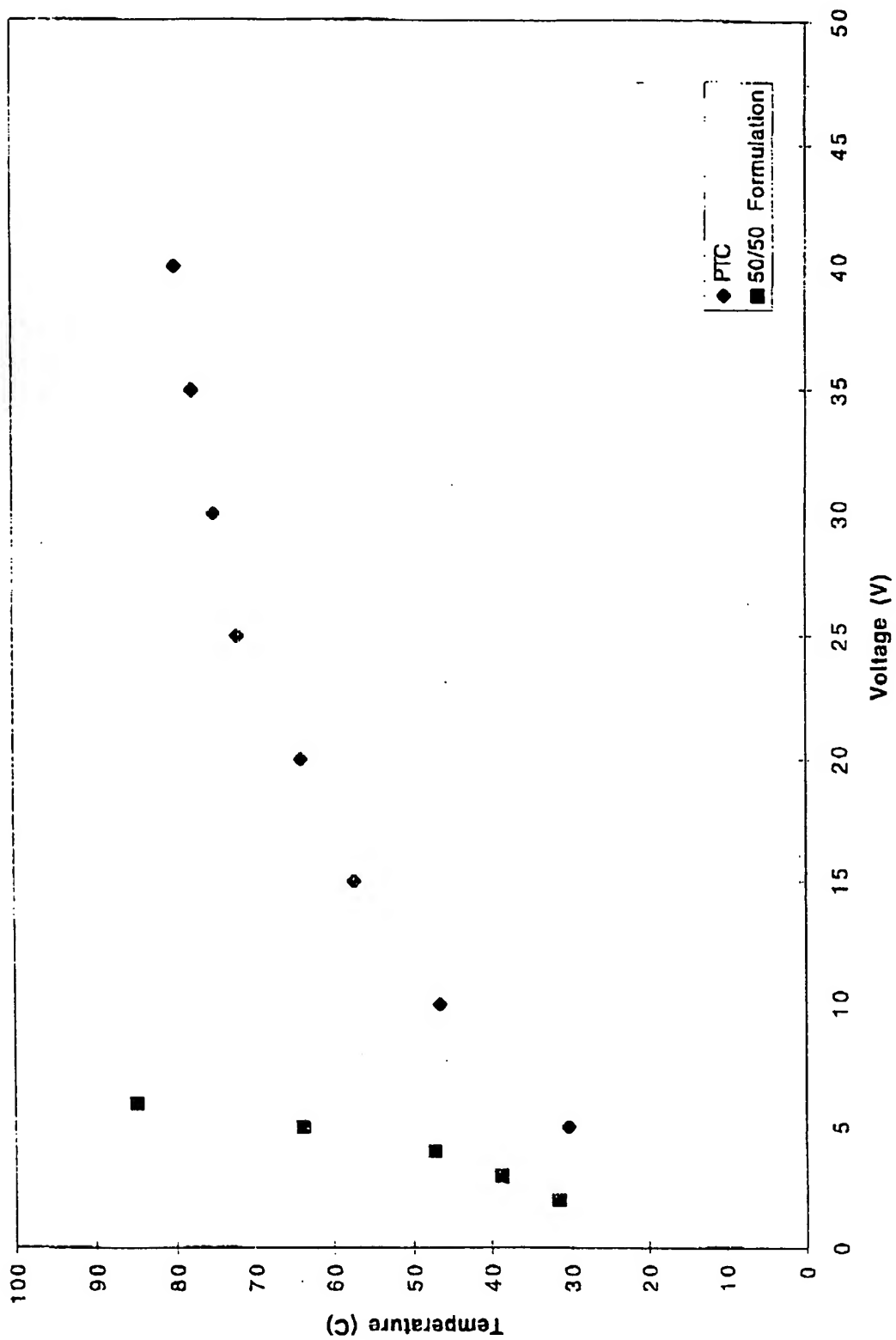


Fig. 33

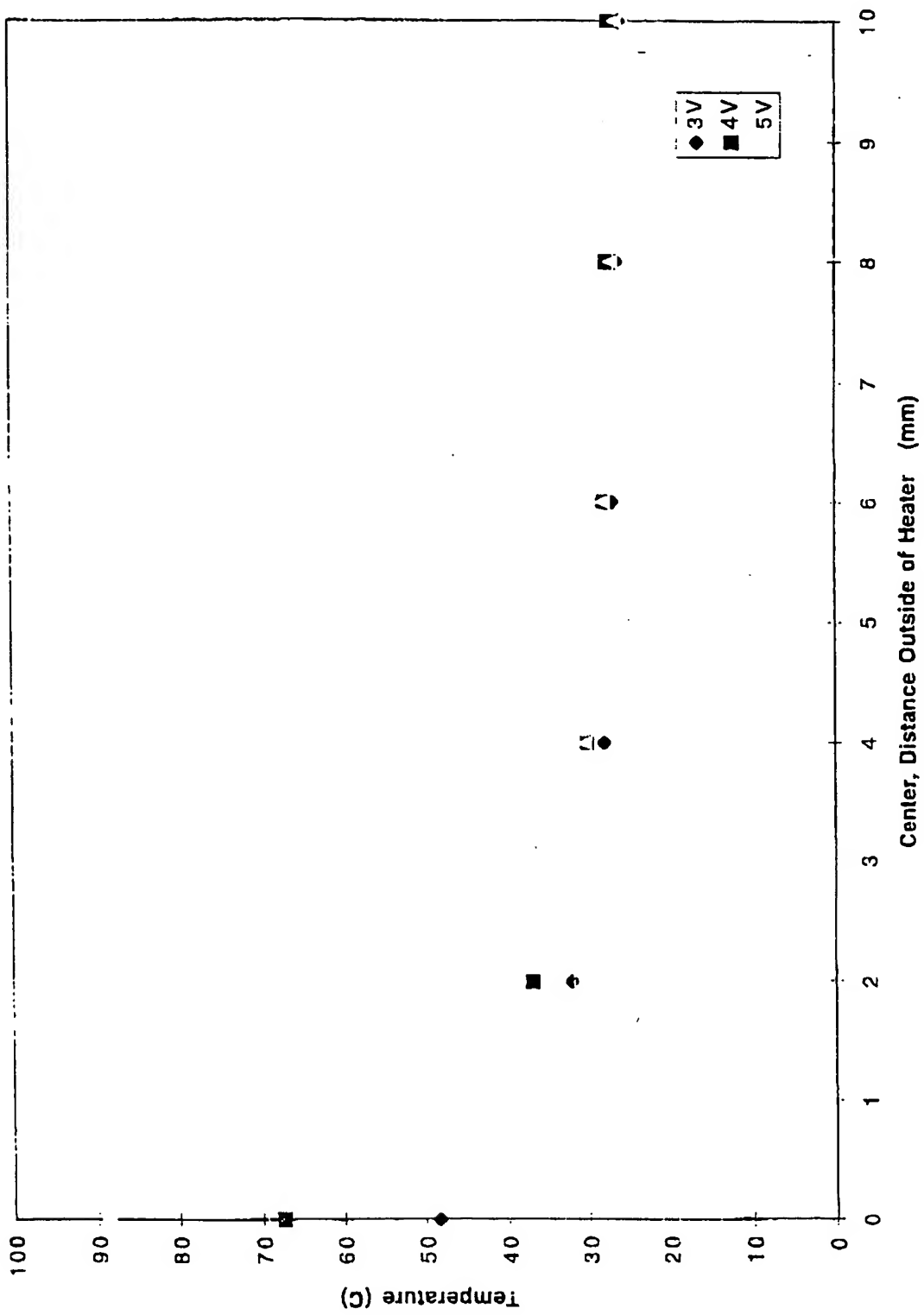


Fig. 34

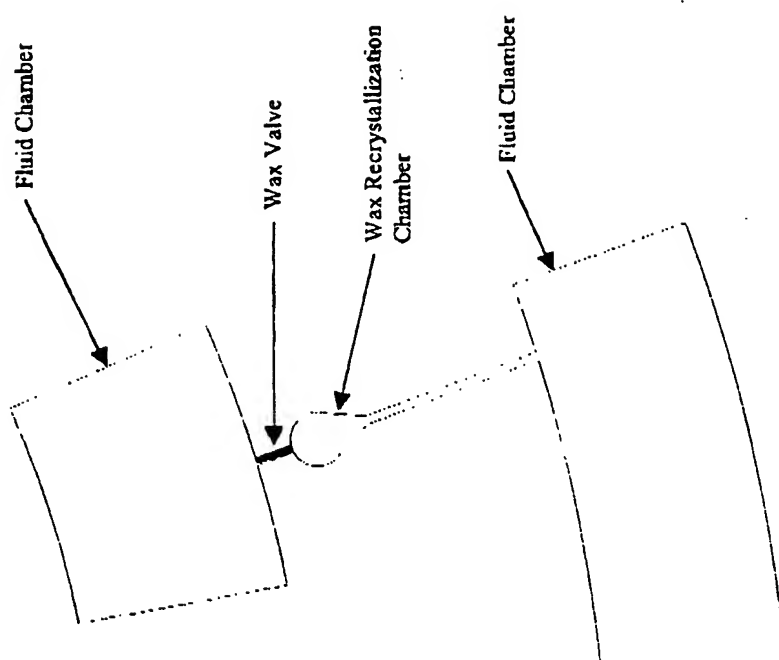


Fig. 35

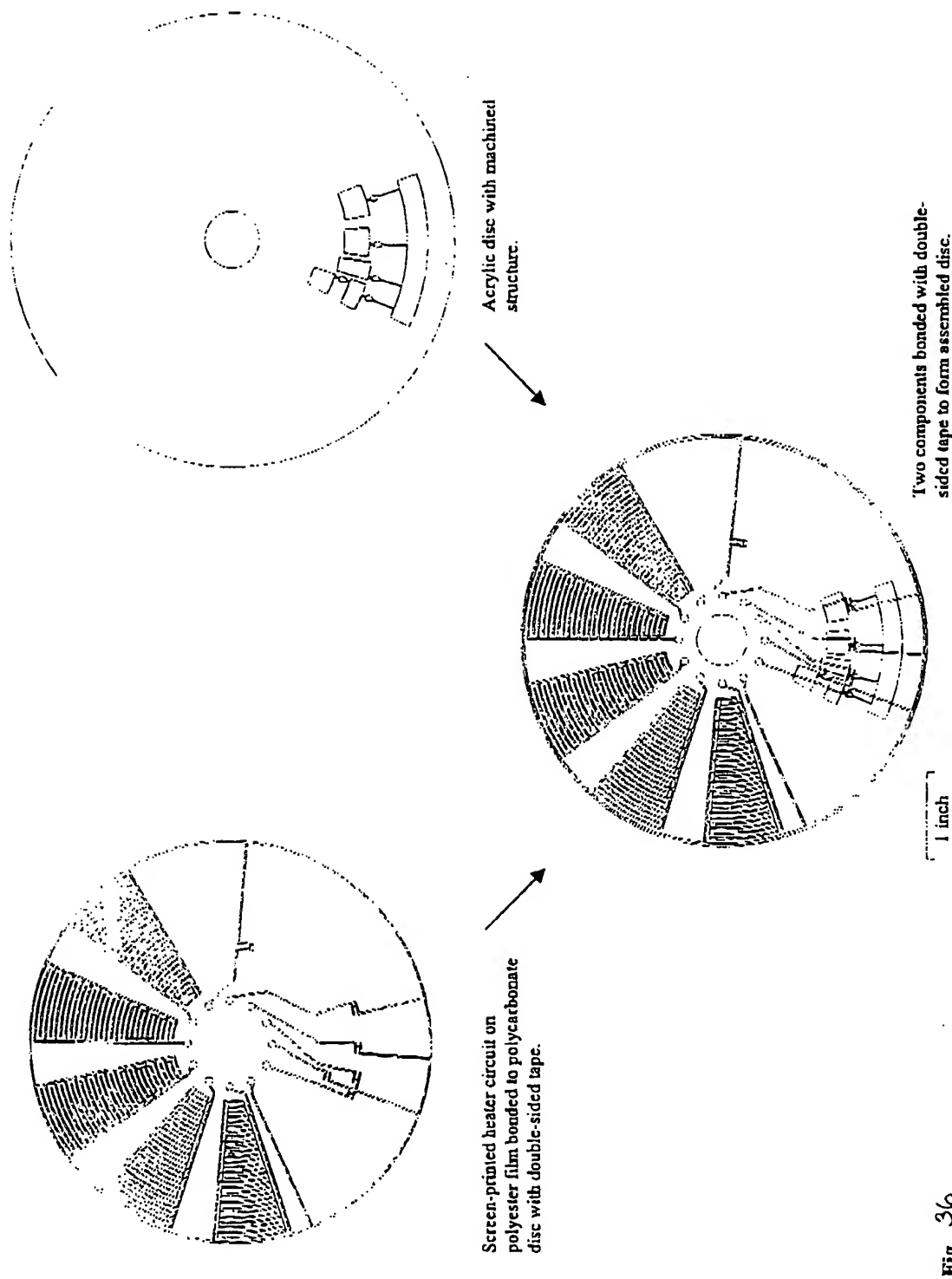


Fig. 36.

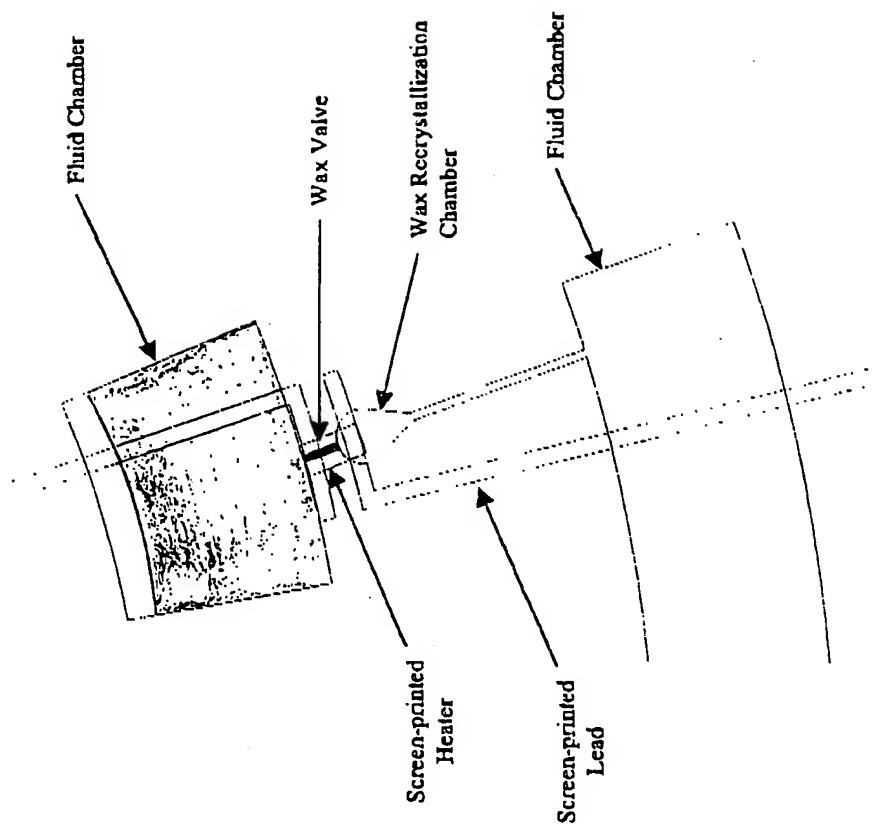


Fig. 37

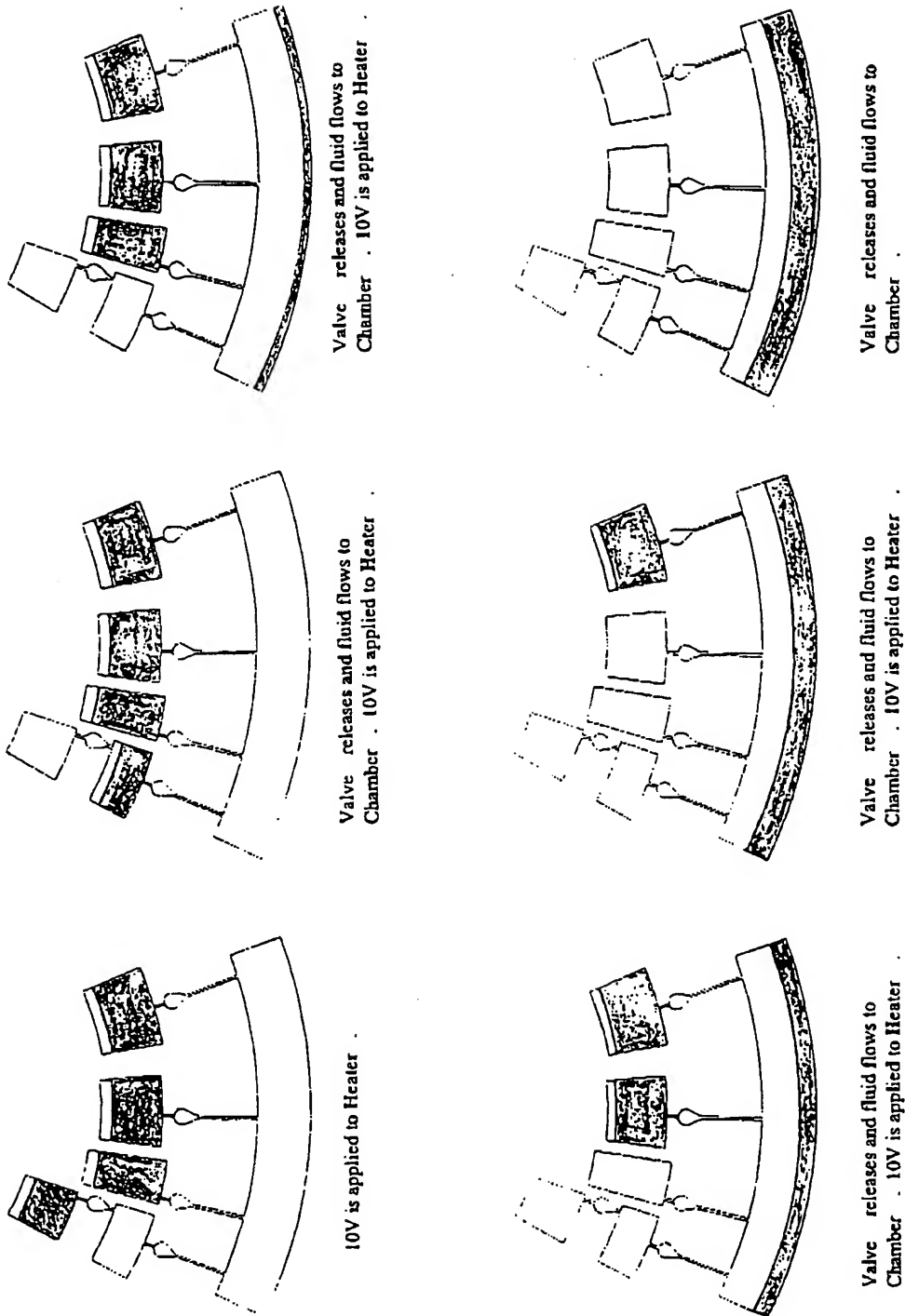


Fig. 38

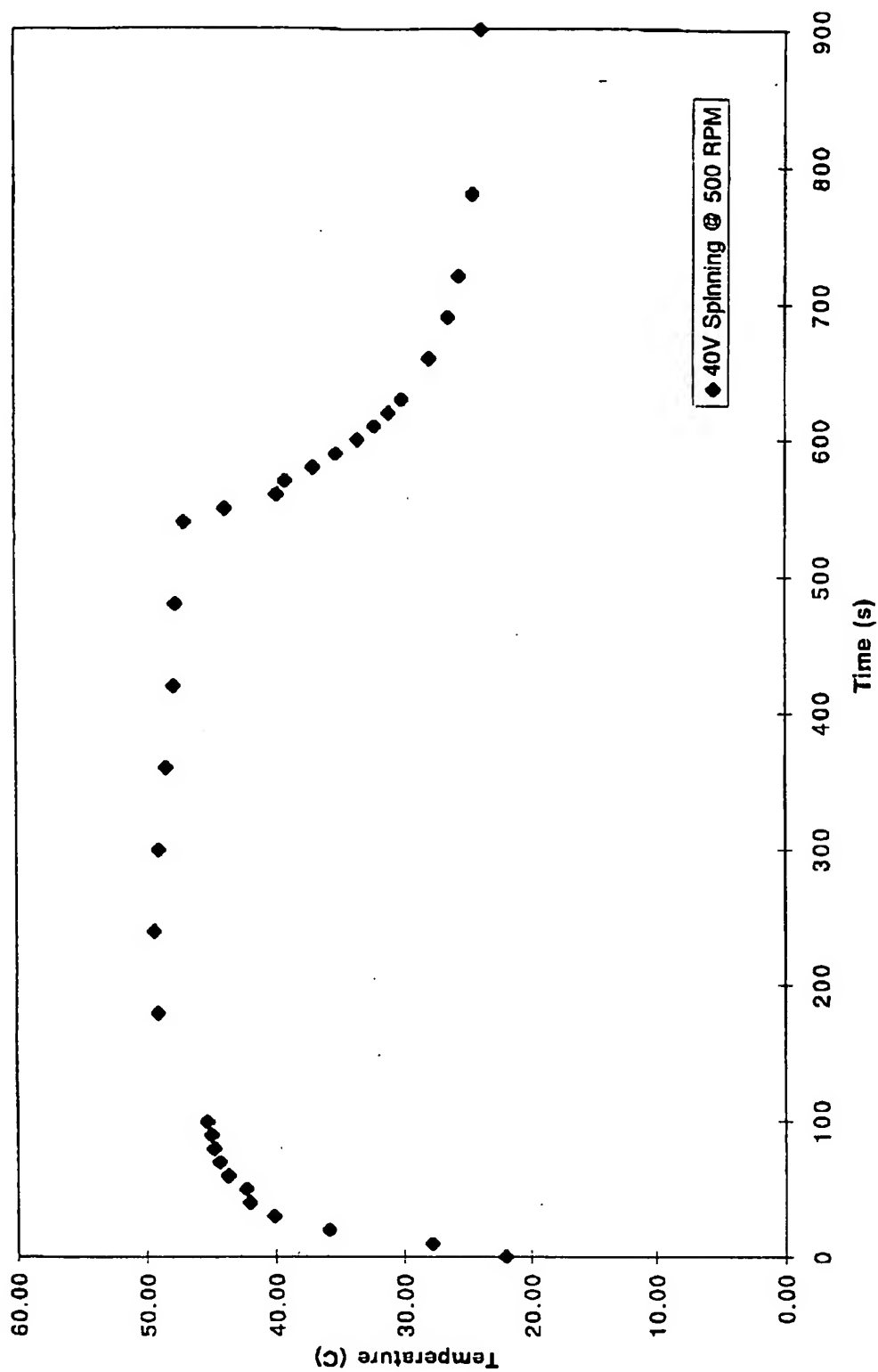


Fig. 39



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>G01N 21/07, F16K 31/00, 13/10,</b> <b>H01C 17/065, H01R 39/64</b>	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 98/53311</b> <b>(43) International Publication Date:</b> 26 November 1998 (26.11.98)
<b>(21) International Application Number:</b> PCT/US98/10520 <b>(22) International Filing Date:</b> 22 May 1998 (22.05.98)  <b>(30) Priority Data:</b> 60/047,488                      23 May 1997 (23.05.97)                      US  <b>(71) Applicant:</b> GAMERA BIOSCIENCE CORPORATION [US/US]; 200 Boston Avenue, Medford, MA 02155 (US).  <b>(72) Inventors:</b> KELLOGG, Gregory; 34 Belknap Street #3, Somerville, MA 02144 (US). KIEFFER-HIGGINS, Stephen, G.; 30 Beaumont Street, Dorchester, MA 02124 (US). CARVALHO, Bruce, L.; 59 Merrill Road, Water- town, MA 02172 (US). DAVIS, Gene, A.; 51 Eldred Street, Lexington, MA 02173 (US). WILLIS, John, P.; 24 Whitney Road, Shirley Center, MA 01464 (US). MINIOR, Ted; 11 Cot Hill Road, Bedford, MA 02155 (US). CHAPMAN, Laura, L.; 87 Marshall Street, Somerville, MA 02145 (US). KOB, Mikayla; 14 Ashford Street, Allston, MA 02134 (US). OELTJEN, Sarah, D.; 46 Hancock Street, Somerville, MA 02144 (US). OMMERT, Shari; 175 Main Street, Medford, MA 02155 (US). MIAN, Alec; 137 Magazine Street, Cambridge, MA 03139 (US).		<b>(74) Agent:</b> NOONAN, Kevin, E.; McDonnell Boehnen Hulber & Berghoff, 32nd floor, 300 South Wacker Drive, Chicago, IL 60606 (US).  <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>  <b>(88) Date of publication of the international search report:</b> 18 February 1999 (18.02.99)
<b>(54) Title:</b> DEVICES AND METHODS FOR USING CENTRIPETAL ACCELERATION TO DRIVE FLUID MOVEMENT IN A MICROFLUIDICS SYSTEM		
<b>(57) Abstract</b> <p>This invention provides methods and apparatus for performing microanalytic and microsynthetic analyses and procedures. Specifically, the invention provides a microsystem platform for use with a micromanipulation device to manipulate the platform by rotation, thereby utilizing the centripetal force resulting from rotation of the platform to motivate fluid movement through microchannels embedded in the microplatform. The microsystem platforms of the invention are also provided having microfluidics components, resistive heating elements, temperature sensing elements, mixing structures, capillary and sacrificial valves, and methods for using these microsystems platforms for performing biological, enzymatic, immunological and chemical assays. An electronic spindle designed rotor capable of transferring electrical signals to and from the microsystem platforms of the invention is also provided.</p> <div data-bbox="841 1178 1398 1913" data-label="Image"> </div>		



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## INTERNATIONAL SEARCH REPORT

Int'l. Application No

PCT/US 98/10520

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 G01N21/07 F16K31/00 F16K13/10 H01C17/065 H01R39/64

According to International Patent Classification (IPC) or to both national classification and IPC

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 07019 A (KIEFFER HIGGINS STEPHEN G ;MIAN ALEC (US); KELLOGG GREGORY (US); G) 19 February 1998 cited in the application see page 48, line 13 - page 51, line 20; figures 3,12,13 ---	1
A	US 5 160 702 A (KOPF-SILL ANNE R ET AL) 3 November 1992 cited in the application see column 5, line 34 - column 7, line 68; figures ---	1-80, 88-91
A	WO 96 07919 A (BIOMETRIC IMAGING INC) 14 March 1996 see page 7, line 1 - line 27 see page 11, line 20 - page 15, line 34; figures ---	1-80, 88-91
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Authorized officer

Hocquet, A

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/10520

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 08556 A (FIRST MEDICAL INC ; ELLSWORTH STOUGHTON L (US); KARUNARATNE ARJUNA) 6 March 1997 see page 4, line 2 - line 30 see page 7, line 14 - line 30 see page 11, line 21 - line 31 see page 15, line 13 - line 32 see page 19, line 23 - line 27 ---	1-80, 88-91
P,A	WO 97 21090 A (GAMERA BIOSCIENCE) 12 June 1997 cited in the application	1
A	see page 23, line 30 - page 24, line 18; claim 25; figures 22,24; examples 5,7	50-70
A	see page 73, line 30 - page 76, line 25; figures 27-29 ---	27-49
A	US 4 258 740 A (KAARTINEN NIILLO H ET AL) 31 March 1981 see column 4, line 44 - line 67; figure 4 see column 6, line 45 - line 57 ---	86
A	US 5 171 533 A (FINE RICHARD A ET AL) 15 December 1992 see column 1, line 63 - column 2, line 9 see column 7, line 59 - column 8, line 29; figures 2-6 see column 11, line 46 - line 66; figures 7-9 ---	86
A	WO 94 26414 A (SYNTEX INC) 24 November 1994 see page 12, line 42 - page 13, line 31; figures 1,2 see page 14, line 5 - line 12 see page 14, line 25 - line 31 see page 18, line 3 - line 5 ---	86
X	US 3 713 062 A (BUTLER S ET AL) 23 January 1973 see column 3, line 23 - line 28; figure 7	81,82
A	see column 3, line 28 - line 33 see column 5, line 64 - column 6, line 46; figure 10 ---	85
X	EP 0 408 207 A (ILLINOIS TOOL WORKS) 16 January 1991 see column 1, line 1 - line 7 see column 3, line 12 - line 16 see column 3, line 39 - line 51; figures 1,2 ---	81,84
	--- -/--	

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/10520

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 722 853 A (BATLIWALLA NEVILLE ET AL) 2 February 1988 see column 1, line 6 - line 61 see column 6, line 52 - column 7, line 9 ----	83
X	US 3 952 116 A (TRENKLER GEORGE ET AL) 20 April 1976 see column 2, line 68 - column 3, line 11 see column 3, line 42 - line 58	81,83
X	see column 4, line 9 - line 14 ----	85
A	GB 1 307 628 A (VER VOLKSEIGENER BETRIEBE ELEK) 21 February 1973 see page 3, line 25 - line 50; figures ----	87
A	US 4 872 821 A (WEISS RONALD R) 10 October 1989 see column 2, line 9 - line 63; figure 2 ----	87
A	FR 1 292 407 A (HERBELIN) 26 September 1962 see the whole document ----	87
A	US 4 030 834 A (BAUER MARTIN L ET AL) 21 June 1977 see column 1, line 35 - line 68 see column 2, line 27 - line 38; figure 1 -----	87

# INTERNATIONAL SEARCH REPORT

Int. l. application No.  
PCT/US 98/10520

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☒ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-80,88-91

microsystem platforms and methods of using them

2. Claims: 81-85

screen printed resistive heater

3. Claim : 86

heat-activated valve

4. Claim : 87

electronic spindle for centrifugal rotor

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/10520

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9807019 A	19-02-1998	AU 4144897 A AU 1283397 A EP 0865606 A NO 982563 A WO 9721090 A	06-03-1998 27-06-1997 23-09-1998 05-08-1998 12-06-1997
US 5160702 A	03-11-1992	NONE	
WO 9607919 A	14-03-1996	US 5627041 A AU 3462795 A CA 2198854 A EP 0778950 A JP 10505672 T	06-05-1997 27-03-1996 14-03-1996 18-06-1997 02-06-1998
WO 9708556 A	06-03-1997	AU 6913196 A EP 0847529 A	19-03-1997 17-06-1998
WO 9721090 A	12-06-1990	AU 1283397 A EP 0865606 A NO 982563 A AU 4144897 A WO 9807019 A	27-06-1997 23-09-1998 05-08-1998 06-03-1998 19-02-1998
US 4258740 A	31-03-1900	US 4248259 A DE 2821258 A FR 2391505 A GB 1596339 A JP 1514142 C JP 54001428 A JP 63063795 B	03-02-1981 23-11-1978 15-12-1978 26-08-1981 24-08-1989 08-01-1979 08-12-1988
US 5171533 A	15-12-1992	AT 119284 T CA 2059659 A DE 69017454 D DE 69017454 T EP 0485493 A ES 2069088 T JP 4507294 T WO 9102238 A	15-03-1995 01-02-1991 06-04-1995 29-06-1995 20-05-1992 01-05-1995 17-12-1992 21-02-1991
WO 9426414 A	24-11-1900	NONE	
US 3713062 A	23-01-1900	NONE	
EP 0408207 A	16-01-1991	US 4931627 A AU 613831 B AU 5478390 A DE 69013996 D DE 69013996 T ES 2063275 T JP 2525937 B JP 3129693 A	05-06-1990 08-08-1991 21-03-1991 15-12-1994 16-03-1995 01-01-1995 21-08-1996 03-06-1991
US 4722853 A	02-02-1988	CA 1277829 A EP 0217512 A IN 167878 A JP 2101212 C JP 8016204 B	18-12-1990 08-04-1987 05-01-1991 22-10-1996 21-02-1996

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/10520

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4722853 A		JP 62039678 A	20-02-1987
US 3952116 A	20-04-1976	NONE	
GB 1307628 A	21-02-1900	NONE	
US 4872821 A	10-10-1989	NONE	
FR 1292407 A	26-09-1962	NONE	
US 4030834 A	21-06-1977	NONE	